Stereochemical studies of H1-receptor histamine antagonists

Mercer, Amanda Denise

Award date:
1989

Awarding institution:
University of Bath

Stereochemical Studies of H_1-Receptor Histamine Antagonists

Thesis

Submitted by Amanda Denise Mercer BSc, MRPharmS,

for the degree of Doctor of Philosophy

of the University of Bath

1989

This research has been carried out in the School of Pharmacy and Pharmacology under the supervision of Dr A F Casy and Prof. C R Ganellin, formerly of SK&F Research Ltd, now at University College, London.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

To my parents

Ad Metam Contendo

ACKNOWLEDGEMENTS

The author wishes to express her grateful thanks to Dr Alan F Casy and Professor C. Robin Ganellin for their initiation, patience, encouragement and helpful advice throughout the course of this work.

To the staff of the Department of Pharmaceutical Chemistry and to her colleagues the author extends thanks for stimulating discussion on many aspects of the work and for their friendship.

Thanks are also due to Mr Harry R Hartell and Mr Dave Wood for skilled ^{13}C- and ^{1}H-NMR spectra.

The author would like to express sincere thanks to Dr Dai Darkin and Mr Nick Viney for their helpful advice and constructive critisism during the time spent at Smith Kline and French Research Limited.

Special thanks are extended to Dr R B Barlow, Department of Pharmacology, University of Bristol, and to Janssen Pharmaceuticals who evaluated the antihistaminic activities of numerous compounds cited in this thesis. Thanks are also extended to Dr M Young, Cambridge University for Binding study work on the resolved compounds and also to Group Captain A Nicholson, Royal Air Force Institute of Aviation Medicine, for the human studies carried out on resolved compounds.

The author also wishes to express thanks to Dr A Drake, Birkbeck College, London for running the Circular Dichroism spectra of the resolved compounds cited in this thesis.

I would also like to thank Lindsay Jacob for her expert typing of the manuscript and Robin Marriott for his help in proof reading.

Finally the author gratefully thanks the Science and Engineering Research Council and Smith Kline and French Research Ltd for financial support throughout the course of this study.

ABSTRACT

Chirality is universally accepted as a paramount feature that governs the biological activity of organic molecules. It therefore has major significance in relation to investigations of the receptors with which pharmacologically active ligands interact and to the design of novel medicinal agents.

Although histamine itself is an achiral molecule, many H_1 receptor antagonists of histamine are either chiral or geometrically isomeric. A review of the stereochemical studies of H_1 antihistamines is given in this thesis to illustrate the marked stereospecifity of the H_1 receptors with respect to antagonists.

The thesis studies differences in antihistaminic potency of enantiomeric pairs of chiral antihistamines at both peripheral and central sites by <u>in vitro</u> (binding and guinea pig ileum) and <u>in vivo</u> (mice and humans) methods and also of novel E and Z pairs of aminopropene-type compounds <u>in vitro</u> and correlates these results with structural and configurational requirements at the H_1 receptor.

The need to resolve a variety of chiral antihistamines, with a high level of optical purity, prior to pharmacological study is emphasized in the work. Work is presented on novel methods of chiral analysis of antihistamines involving ^1H NMR and/or HPLC techniques with cyclodextrins or α-acid glycoprotein since the most commonly employed methods eg optical rotation do not provide an 'absolute' measure of optical purity.

From the human study data the results indicate that the sedative side effects associated with H_1 antagonists are due to blockade of central H_1 receptors. Correlation of the structural requirements for antagonistic activity at histamine receptors highlights configurational similarities between the semi rigid aminopropenes and the more flexible pheniramine and diphenhydramine types.

CONTENTS

Chapter 1

Introduction

1.1 INTRODUCTION

Chirality is universally accepted as a paramount feature that governs the biological activity of organic molecules. It, therefore, has major significance in relation to investigations of the receptors with which pharmacologically active ligands interact. The main reasons for studying the stereochemistry of drug molecules is the information that such data can provide with respect to the nature of drug-receptor interactions and receptor characteristics. Prior to reaching the receptor site, the drug molecule is subjected to a variety of physiological processes such as absorption, distribution, metabolism, uptake at storage sites and excretion. Many of these processes may be stereoselective (eg enzyme reactions) to varying degrees resulting in differing isomeric concentrations at the receptor sites; in these cases the isomeric potency ratios may be due to concentration effects rather than drug-receptor interactions.

Steric effects are likely to influence penetration of membranes by drugs if active transport mechanisms are involved. The stereospecificity of amino acid and carbohydrate uptake by the small intestine and by bacteria has been studied and results show the R amino acids to be absorbed much more rapidly than the corresponding S-enantiomer.

Catecholamines provide good examples of differences in the uptake and binding of enantiomers. Levo isomers of octopamine, m-octopamine and norepinephrine are preferentially retained by the heart.

Nonsteroidal anti-inflammatory agents such as ibuprofen and naproxen also show differences in potency and metabolism between their isomeric forms.

In these cases, the S isomers are the active forms - the inactive R enantiomers are activated in the body by their conversion into S enantiomers (Wainer and Doyle, 1984).

Examples of enantiomeric pairs in which each isomer shows considerable differences in its biological effects have long been known. The first recorded case was due to an observation of Louis Pasteur. In 1858 Pasteur reported that when the mould Penicillum glaucum fed on a mixture of enantiomeric tartaric acids it consumed only the dextro (+) enantiomer and left the levo (-) tartaric acid behind.

Since this time, there have been many reported cases of enantiomeric differences in pharmacological activity (Williams and Lee, 1985; Casy, 1970).

The example of thalidomide, where the (+) enantiomer provides the desired hypnotic effects and the (-) isomer is responsible for the well known teratogenic effects of the racemic agent. (Karnes and Sarkar, 1987), highlights the need for chiral resolution of drug compounds - discussed later in the thesis.

This thesis looks in more detail at antagonists of the H_1 receptor of histamine and studies the stereochemical aspects associated with a selection of these compounds.

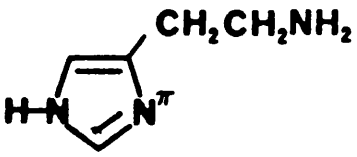
Since histamine has many well defined effects it may be assumed that the biological response is via a receptor or receptors.

The effects of histamine are considered to be mediated by at least three sets of receptors ie. H_1, H_2 and H_3.
Those effects mediated by the H_1 receptor include contraction of smooth muscle and the dilatation and increased permeability of the capillaries. The effects of histamine on vascular smooth muscle are mediated by H_2 as well as H_1 receptors.

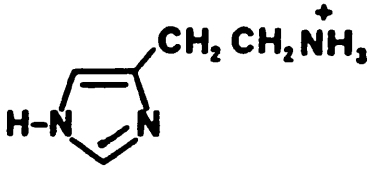
Other effects which are mediated by H_2 receptors include cardiac accelerating effects and the stimulating action of histamine on the secretion of gastric acid. Two examples of H_2 antagonists are cimetidine and ranitidine, both of which are achiral. The third subclass of histamine receptors appear to be involved in the feedback control of histamine synthesis and release in the brain (Arrang et al, 1983). This thesis does not discuss these latter types of histamine receptors or antagonists in any detail.

1.2 Introduction to Antihistamines

Histamine is an achiral molecule (1a) which exists under physiological conditions as the monocation (1b) in equilibrium with its $N^{\pi}H$ tautomer (Ganellin, 1982).



(1a)



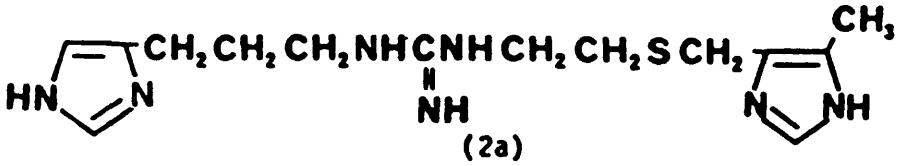
(1b)

A variety of chiral analogues produced by introduction of a methyl group into the ethylamino side chain have been tested as H_1 and H_2 agonists but all proved to be of much lower potency than that of the parent compound (Ganellin, 1982).

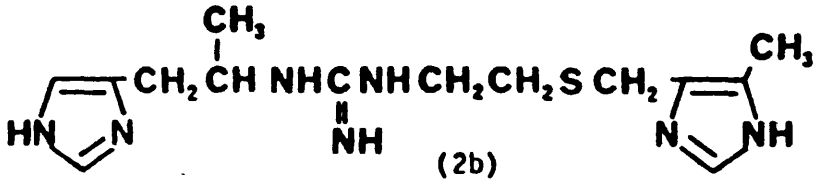
At the H_3 receptor, however, one analogue ie R-α-methylhistamine (1c) was shown to be 15 times more potent than histamine itself and 100 times more potent than its S isomer (Arrang et al, 1988).

(1c)

Stereoselectivity has also been reported for compounds acting at H_2 receptor sites (Elz and Schunack, 1988; Arrang et al, 1985). By replacing the homohistamine side chain of the potent achiral H_2 agonist impromidine (2a) with α-methyl histamine yields sopromidine (2b) and its S enantiomer



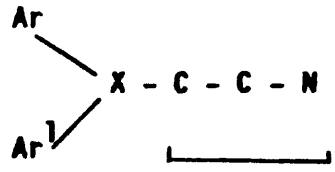
(2a)



(2b)

Sopromidine acts as a highly potent H_2 agonist (7 times that of histamine) whereas its S enantiomer acts as an H_2 antagonist. In contrast, at the H_3 receptor both compounds act as an antagonists having apparent dissociation constants very similar to impromidine. These results show that the H_3 receptors are chemically stereoselective, with structural requirements different from those of H_1 and H_2 receptors.

Most compounds that are effective at low dose levels in antagonizing histamine at H_1 receptor sites (Ash + Schild, 1966) may be described by the general structure (3) where Ar is aryl (phenyl, substituted phenyl or heteroaryl) and Ar^1 is a second aryl group or Ar-CH_2 group.



β-aminoethyl side chain

(3)

The unit X may be nitrogen, saturated (sp^3) carbon-oxygen (ether linkages) or a saturated carbon linked directly to the β-aminoethyl side chain. X-C may also be replaced by a pair of alkenic (sp^2) carbon atoms ie. a C=C double bond. The terminal nitrogen is part of a tertiary acyclic or alicyclic basic group eg. dimethylamino (-NMe_2) or 1-pyrrolidino (-N⬠). Tricyclic derivatives in which the two aromatic rings are bridged are also encountered and these do not differ essentially from the general structure. Overall, H_1-antagonists have structures comprising a double - aromatic unit linked by a two or three atom chain to a tertiary amino basic group. Histamine, itself differs from its antagonists in possessing only a single aromatic (imidazole) feature and having a primary (NH_2) basic feature.

In presenting the stereochemical features of non-symmetric analogues of (3) it is convenient to clasify them into distinct groups.

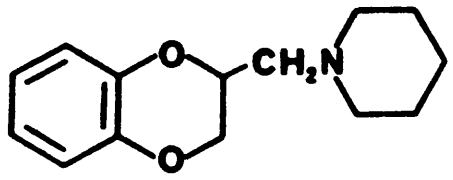
ie. 1. Chiral and

2. Geometrically isomeric antihistaminic groups.

1.3 Chiral Antihistaminic Agents

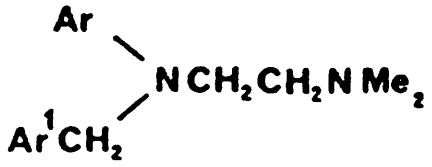
1.3.1 Early Examples and Ethylenediamines

It is interesting that the first compound reported capable of antagonising some of the effects of histamine in animals was the chiral 1-4 benzodioxane (4) (Fourneau and Bovet, 1933). It had



(4)

only weak activity and was examined solely as the racemate. These early leads led to the development of phenbenzamine (5), mepyramine (6) and many related ethylenediamine derivatives (Review, Casy, 1978).



(5) Ar = Ph

Ar^1 = Ph

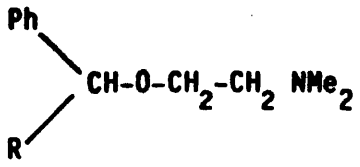
(6) Ar = 2-Py

Ar^1 = 4-MeO-C_6H_4

No chiral analogues of phenbenzamine have been described.

1.3.2 Tertiary Amino Alkyl Ethers

During the 1940's, medicinal chemists in the USA investigated antihistamines in which the alkylamino nitrogen of phenbenzamine (5) was replaced by oxygen to give a series of basic ethers. This led to the introduction of the prototype member of this group, diphenhydramine (7), several chiral variants of which have been examined. The simplest, obtained by para methyl substitution



7) R = Ph

8) R = pMePh

of one of the phenyl rings, is the potent racemate (8) marketed as neobenodine.

Jarrousse and Regnier (1951) first reported the antipodal forms, after resolution of the carbinol intermediates as phthalate esters with quinine. The relative potencies of these enantiomers in antihistamine tests were as follows (Table 1.1).

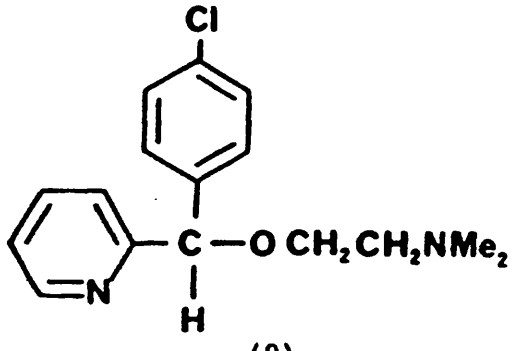
Test	(+)	(-)	RS
Inhibition of histamine-induced contractions of guinea-pig ileum	1	0.37	0.75
Protection of guinea-pig against histamine induced bronchospasm	1	0.25	0.5
Protection of guinea-pigs against a lethal i.v. dose of histamine	1	0.11	0.79

Table 1.1 Relative potencies of the isomers of neobenodine in three antihistamine tests.

Later work (Rekker et al 1971 and 1972), using optical isomers of specific rotations R +13.0° and S -10.3° in ethanol (Stelt et al, 1969) reported that the R(+) form (pA_2 8.7) was 65 times more potent than the S(-) form (pA_2 6.9) in antagonizing guinea pig ileum sites. The R and RS forms unexpectedly displayed equal potencies. These results show higher potency ratios than those obtained in the French work, probably due to the poor optical purity of the dextro isomer used originally.

The compound (8) is used clinically as the racemate. Antipodal forms of <u>para</u>-ethyl and methylamino analogues of (8) differ similarly in their antihistaminic potencies in the guinea pig ileum assay (Nauta and Rekker, 1978). The <u>ortho</u> methyl analogue, orphenadrine, is only 1/10th as active as (8) and its antipodes are equi-active (it is used as an anticholinergic agent).

Carbinoxamine (9) is another chiral analogue of diphenhydramine,



(9)

and in this case 2-pyridyl and 4-chlorophenyl are the two non-identical aryl groups.

Antipodal forms obtained by resolution with d and l tartaric acids differed significantly in activity (Roszkowski and Govier, 1959) - as shown in Table 1.2.

Table 1.2 : Relative potencies of RS carbinoxamine and the individual enantiomers

	Relative Potencies	
Isomer	Test 1	Test 2
(-)	29 (ED_{50} 0.26μg/100ml)	34 (ED_{50} 50μg/kg)
(+)	1	1
(±)	15	19 (ED_{50} 90μg/kg)

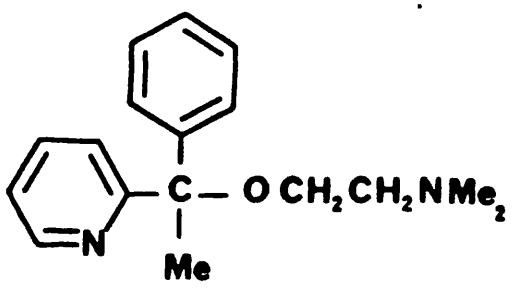
Footnotes for Table 1.2.

Test 1. Inhibition of supramaximal histamine - induced contractions of guinea pig ileum.

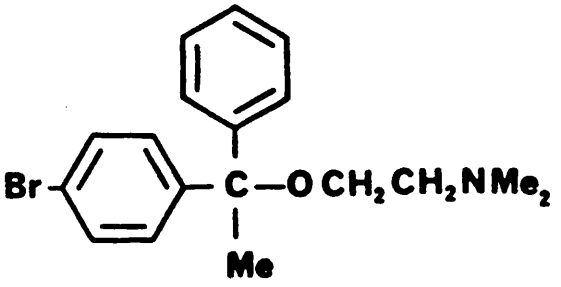
Test 2. Protection of guinea pigs against lethal i.v. histamine (3mg/kg) given 20 mins after i.p. carbinoxamine.

Barough et al (1971) showed the more active levo isomer of (9) to have the S-configuration and to be superimposable on the more active dextro enantiomers of the pheniramines (17, 17b and 17c) (Shafi'ee and Hite, 1969).

Doxylamine (10) and mebrophenhydramine (11) are chiral analogues of carbinoxamine - both having a methyl substituent on the benzylic carbon atom.



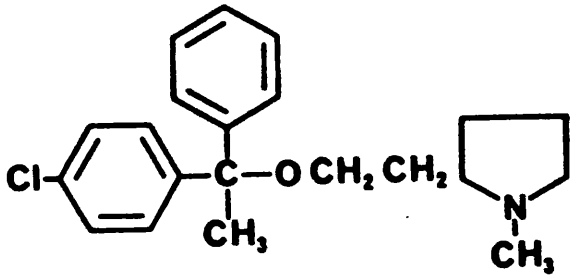
(10)



(11)

Neither of these racemates have been reported to have been resolved. Doxylamine is used clinically as the racemate (Martindale, 1982) but mebrophenydramine was not marketed (Novak and Protiva, 1959) due to toxic side effects.

Clemastine (12) is similar in structure to mebrophenhydramine (11), differing only in the halogen substituent on the aromatic ring and also the aminoalkyl side chain terminating in an alicyclic basic group.



(12)

The compound (12) possesses two chiral centres - one at the benzylic carbon and the other at C-2 of the pyrrolidine ring. Clemastine, therefore exists in four optically active forms, all of which have been separated and tested for pharmacological activity. The absolute configurations were established by degradation to R and S 1-methyl-2-pyrrolidinoethanol and by X-ray analysis of the most active isomer (Ebnother and Weber, 1976). The pharmacological data are given in Table 1.3.

Table 1.3 : Antihistaminic activities of clemastine (12) and its isomers

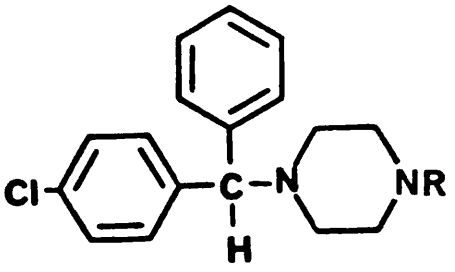
Isomer[1]	Prevention of Histamine Toxicity[2] ED_{50} mg/kg sc	Prevention of Histamine Spasm[3]	pA_2 [4]
RR (clemastine)	0.04	ca. +7	9.45
SS	5.0	ca. -1.5	7.99
SR	11.0	ca. -6	8.57
RS	0.28	ca. +5	9.40

Footnotes:

1. Configuration of benzylic centre followed by that of C-2 of the pyrrolidine ring.
2. Dose which protects 50% of a population of guinea pigs from the lethal effects of a 20mg/kg s.c. dose of histamine given 3h after receipt of the test substance. Animals surviving 12h were regarded as protected.
3. Potency relative to that of the standard drug thenalidine (=1) in the guinea pig ileum test. Spasms induced by 5×10^{-8} g/ml histamine HCl applied 5 min after addition of test substance.
4. Guinea pig ileum data obtained by Nauta and Rekker, (1978).

From Table I.3, it is clear that the chiral centre at the benzylic carbon is of greater importance with respect to pharmacological activity since the RR and RS isomers are the most active. Many other pharmacological and clinical studies of clemastine have been carried out but none of these provide comparative data upon its isomers.

Several antihistaminic piperazines (cyclizines) possessing a chiral substituted benzylic feature are known. (P'An et al, 1954).



R

(13) $CH_2C_6H_4$2-Me

(14) $CH_2C_6H_4$4-But

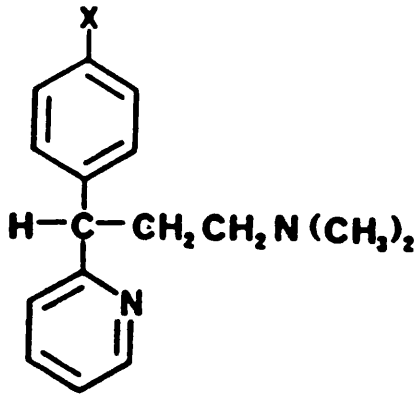
(15) $CH_2CH_2OCH_2CH_2OH$

(16) $CH_2CH_2OCH_2COOH$

These include meclizine (13), buclizine (14), hydroxyzine (15) and cetirizine (16). Two compounds (13) and (14) are well known clinical agents but all reports on these compounds relate to the racemic mixtures only. Cetirizine (16) has recently been launched as a non-sedating antihistaminic agent .

1.3.3 3-Amino-1-aryl-1-(2-pyridyl) propanes (Pheniramines)

This group provides three compounds in clinical use, ie. pheniramine itself (17), brompheniramine (17b) and chlorpheniramine (17c). The halogen derivatives are distinctly more potent than pheniramine itself. The structural resemblence of the group to the 2-pyridyl congeners of diphenhydramine (7) is clear, and the compounds are also saturated analogues of triprolidine (23) and related amino propenes.



X = H (17)

X = Br (17b)

X = Cl (17c)

Resolution of all three compounds as diastereomeric salts of phenylsuccinic acid has been reported (Patent, 1960). The patent claims that the (+) isomers have enhanced antihistaminic activity substantially free from untoward side effects - while the (-) isomers have local anaesthetic and psychotherapeutic uses.

Several reports relate to the comparative pharmacology of (+) and (-) chlorpheniramine (17c). An in vitro study (Roth and Govier, 1958) showed the dextro isomer to have an antihistaminic potency of approximately twice that of the racemate and 200 times that of the levo isomer (guinea pig ileum test, dose of maleate required to produce 50% inhibition of muscle spasm induced with histamine - 0.2µg/ml; (+) 0.8µg/ml; RS 1.7µg/l; (-) 190µg/l).

In an in vivo test (protection against i.v. or aerosolized histamine lethalility) the dextro isomer had 2-3 times the potency of the racemate and 100 times that of the levo. The central nervous system effects of all three forms of chlorpheniramine in mice and cats were similar - all producing general stimulation while no signs of sedation were observed.

Brittain et al (1959) found a more modest potency ratio between chlorpheniramine enantiomers at guinea pig ileum sites and reported pA_2 values (+) 8.47, RS 8.10, (-) 7.81 measured after a 2 minute contact time. More recent pA_2 values of chlorpheniramine enantiomers determined at guinea pig ileum sites are listed in Table 1.4 (Nauta and Rekker, 1978). These indicate that the potency ratio of Brittain et al, (1959), is too low - possibly because of the contact time used.

Table 1.4 : In Vitro pA_2 values of chlorpheniramine isomers obtained on guinea pig ileum

Enantiomer	pA_2 [1]	pA_2 [2]
(+) chlorpheniramine	9.3	9.3
(−) chlorpheniramine	7.8	7.5

Footnotes:

1. Data from Jansenn Pharmaceutica, in Rekker et al, 1971 and 1972.
2. Data from Gist-Brocades in Rekker et al, 1971 and 1972.

Subsequently Roth (1961) provided data on the three forms of pheniramine (17) and its bromo analogue (17b) (Table 1.5).

Table 1.5 : In vitro antihistaminic potency of RS, (+) and (-) isomers of pheniramine and brompheniramine on the guinea pig ileum

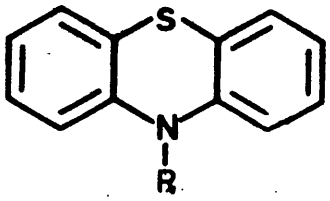
Compound	Form	ED_{50} µg/l	Relative Potency (RS = 1)	pA_2 (Nauta and Rekker, 1978)
Pheniramine (17)	RS	9.0	1.0	7.8
	(+)	5.5	1.6	7.5 (7.96)
	(-)	170.0	0.05	6.0 (6.74)
Brompheniramine (17b)	RS	1.4	1.0	-
	(+)	0.56	2.5	-
	(-)	88.0	0.016	-

In oral tests in guinea pigs the therapeutic indexes (LD_{50}/protective $dose_{50}$) of the (+) isomers of (17c) and (17b) were about twice that of their corresponding racemate and many times that of the levo isomer eg. chlorpheniramine, (17c), RS 1430, (+) 3380, (-) 25. Although clinical use of the more potent pheniramines has not demonstrated a significant separation of sedative activity from an effective antihistaminic response - the reduced dose of these isomers, relative to the racemic dose, results in a corresponding reduction of sedative side-effects. Dexchlorpheniramine and dextro-brompheniramine have both been marketed for clinical use in the USA.

The configuration of the dextrorotatory pheniramines has been shown to be S by chemical methods (Shafi'ee and Hite, 1969) and confirmed by X-ray crystallography (James and Williams, 1974).

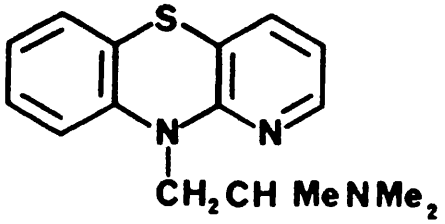
1.3.4 Phenothiazine Derivatives

A number of antihistaminic agents have been discovered as a result of bridging the terminal diaryl unit of phenbenzamine (5) and diphenhydramine (7) across the ortho positions of the aromatic rings. The best known examples of antihistamines so derived are phenothiazine derivatives (18), where the bridging



(18)

group is sulphur and the nitrogen of the ring system is substituted with an alkylamino chain (R) similar to that found in other classes of antihistamines. Promethazine (18, R = $CH_2CHMeNMe_2$) and its 1-aza analogue isothipendyl (19) both contain a chiral side chain and have been resolved.



(19)

Enantiomers of promethazine (18) have similar antihistaminic and pharmacological properties (Toldy et al, 1959). Results with antipodes of isothipendyl produced unexpected results since both (+) and (-) forms were less potent than the racemate in an in vivo test. The levo isomer, however, was about one half as potent as the dextro form (Table 1.6). In the in vitro ileum test, the antipodal forms had similar activities that were marginally greater than that of the racemate.

Table 1.6 : Antihistaminic potencies of RS, (+) and (-) isothipendyl (19) in the guinea pig

Form	No. of Animals	Protective dose$_{50}$ against i.v. histamine[a] mg/kg	Relative Potency (RS=1.0)	In Vitro test (ileum)[b] Effective conc. 50µg/l
RS	30	0.86 (0.54-1.37)	1.0	0.76
	29	0.90 (0.5-1.62)	-	-
(+)	30	1.2 (0.81-1.77)	0.7	0.7
(-)	29	2.3 (1.15-4.6)	0.37	0.68

Footnotes:

a. 1.1mg/kg histamine 2HCl

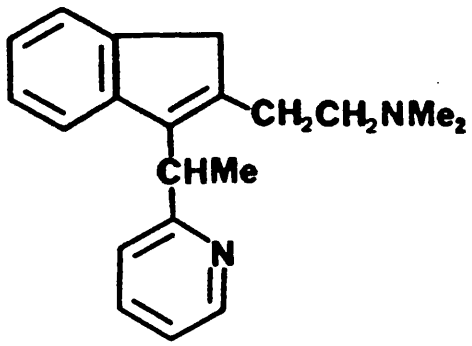
b. Four tests carried out

95% confidence limits are given in parentheses

It is important to note here that the chiral centres of these two phenothiazines are much further away from the aromatic centres than the chiral carbons in the diphenhydramines or pheniramines. This factor appears to be very important for receptor sensitivity to ligand stereochemistry. The results quoted previously (Table 1.3) for clemastine (12) support this view.

1.3.5 Indene Derivatives

There are two indene derivatives that are important as antihistaminics ie. dimethindene (20) and phenindamine (21). The first of these, dimethindene (20) has been resolved as the tartrate salts and the pharmacological activity found to be greatest in the levo isomer, which is about four times more potent than dexchlorpheniramine (Huebner et al, 1960; Barrett et al, 1960).



(20)

Borchard et al (1985) obtained the following data from isolated guinea pig organs, suspended in an organ bath at 31°C (Table 1.7).

Table 1.7 : *In vitro* antihistaminic potencies of the enantiomers of dimethindene (20)

Organ[a]	pA_2 values (-)	(+)	(+)/(-) Potency ratio
Left atria[b]	9.4	6.3	1600
Trachea	9.3	7.0	200
Aorta	9.9	7.3	400
Ileum	9.1	7.8	20

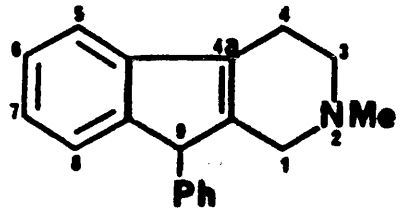
Footnotes:

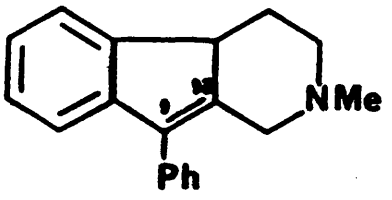
a. Preparations stimulated with histamine or 2-pyridylethylamine (trachea only).

b. Electrically driven at 1 Hz.

A 90 minute washout of levo dimethindene led to a small reduction in receptor blockade in the ileum and aorta whereas there was nearly no change in the left atria and trachea. The antagonistic action of the dextro isomer was almost completely reversed in the aorta and ileum by the same treatment, but only to a small extent in the left atria and trachea. Amongst these tissues, ileum sites appear to be the least stereoselective (differing equilibrium rates may contribute, however, to variations in the antipodal potency ratios).

Although the second chiral indene derivative phenindamine (21) is used clinically as the tartrate salt (Thephorin), the material is a diastereomeric mixture (60:40 approx) (Kern, private comm.) and no data on optically pure materials are available.





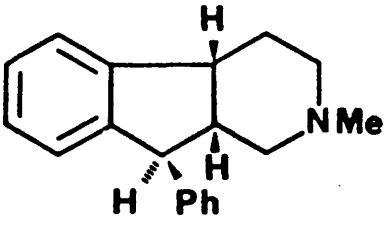
pA_2 8.8

(21a)

(21b)

The freshly synthesized base is a mixture of 4a-9a (21a) and 9a-9-ene (21b) positional isomers from which the more active 4a-9a-ene may be separated as a tartrate salt (most other salts are 9-9a enes). These structural relationships have been established by high field NMR (^1H, ^{13}C) investigation (Hussain, 1987; Branch et al, 1988).

Dihydroanalogues of phenindamine are less active than the parent and the more potent of the two reported probably has the stereochemistry (22) (Augstein et al, 1972).



pA_2 7.6

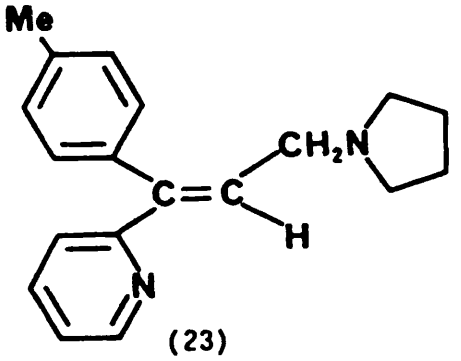
(22)

1.4 Geometrically Isomeric Antihistaminic Agents

1.4.1 1,1-Diaryl-3-Amino-propenes

Antihistaminic agents of this type are used clinically as single isomeric forms. Since geometrical isomers usually differ in their physical properties they may be separated relatively easily without the need for use of chiral agents.

The best known aminopropene is triprolidine (23), E-1-(2-pyridyl)-3-(1-pyrrolidino)-1-(4-tolyl)prop-1-ene (Adamson et al, 1951 and 1957).

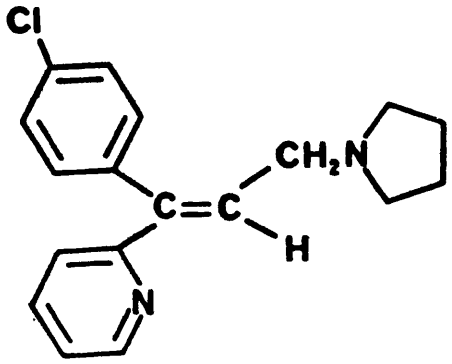


(23)

No clinical comparisons of triprolidine (23) and its corresponding Z-isomer appear to have been carried out; this is not surprising in view of the large potency difference between the two isomers found in animal tests.

E/Z pairs of these compounds may be separated from binary mixtures by fractional crystallization of hydrogen oxalate or hydrochloride salts. It was later found that the ratio of isomeric propenes was dependent on their equilibration rates. In the case of the triprolidine pair, equilibration in acid yields the desired E-isomer almost exclusively (Ison and Casy, 1971).

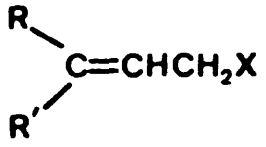
Adamson et al (1951) reported differences in antihistaminic activity for geometrical isomers but specific examples were limited to the 4-chloro analogue (24) and its isomer.



(24)

In the usual guinea pig ileum test the E isomer was 80 times more active than the Z form. Further pharmacological studies (Ison et al, 1973) confirmed the high activity of triprolidine (23) (log K_b 9.95) and the superiority of E over the corresponding Z isomer as antihistaminic agents. In four such pairs the E-isomer had about 10 times the affinity of its Z partner for histamine sites (Table 1.8).

Table 1.8 : Comparative affinities of 3-aminoprop-1-ene isomers



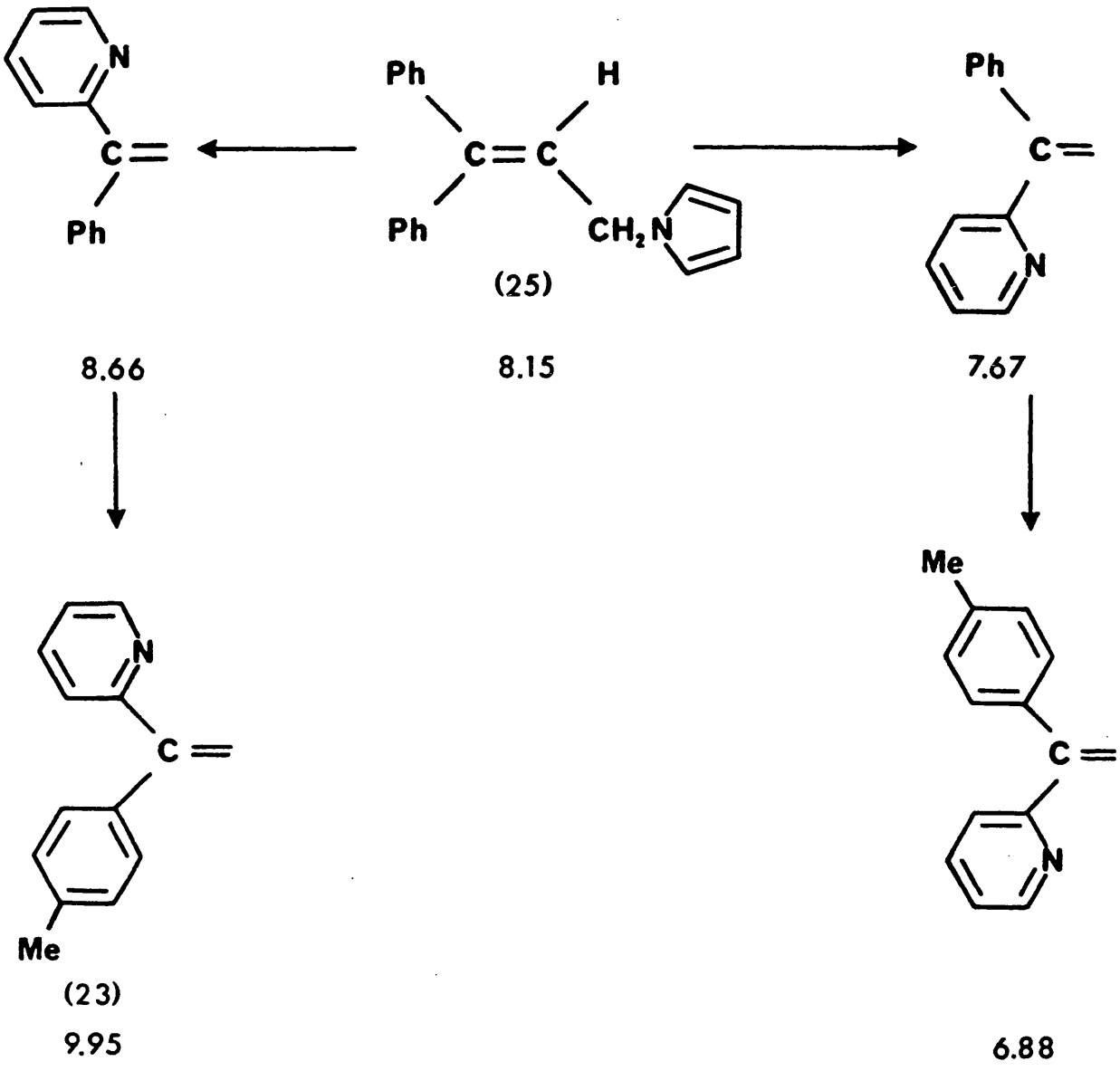
R	R^1	X	Δ log K_b	Ratio of affinities (E/Z)
2-pyridyl	p-tolyl	NC_4H_8	3.07	1170 (23)[a]
2-pyridyl	phenyl	NC_4H_8	0.97	9.3
2-pyridyl	p-Cl.C_6H_4	NC_4H_8	0.83	6.8a (24)
2-pyridyl	phenyl	NMe_2	1.2	16
phenyl	p-Cl.C_6H_4	NC_4H_8	1.1	13

Footnote:

a. Earlier potency ratio quoted as 80 (Adamson et al, 1951).

The affinity ratio for triprolidine and its isomer (1170) was remarkably higher than those of other pairs.

Taking the diphenyl compound (25) as the standard it is seen (Scheme 1) that replacement of phenyl by 2-pyridyl raises the affinity constant when E-to-the pyrrolidino group but lowers it when Z to the same group. In contrast, the effect of substitution of phenyl by p-tolyl are opposite; in the position trans to the pyrrolidino group it reduces activity and in the cis it enhances it. (Scheme 1).

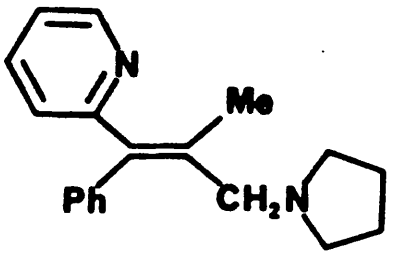


Scheme 1: Log K_b values for triprolidine and its analogues.
(Ison et al, 1973)

In the case of phenyl and p-chlorophenyl the effects are much less.

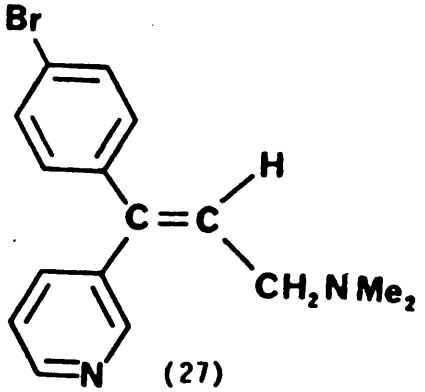
One of the causes of the large affinity ratio observed for triprolidine (23) and its Z-isomer must therefore be due to the combination of both 2-pyridyl and p-tolyl groups being in favourable positions relative to the amino group in the active E-isomer and unfavourable positions is the less active Z compound.

This view that a coplanar aromatic-double bond system in 1,1, diaryl-3-aminopropenes is important for antihistaminic activity (Casy and Ison, 1970) was supported by the fact that a 2-methyl analogue (26) of triprolidine (23) in which a coplanar conformation, as described previously, is unfavoured, has a very low affinity for histamine H_1 receptors (Ison et al, 1973).



(26)

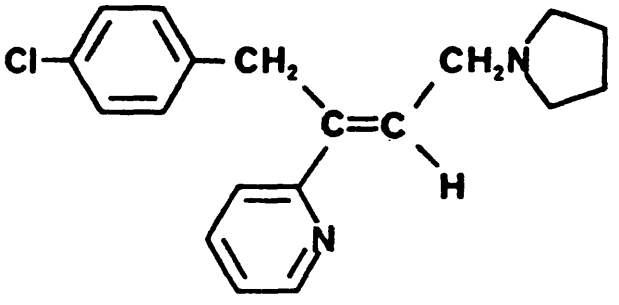
Zimeldine (27) and its corresponding E-isomer are 3-pyridyl analogues of triprolidine. Stereoselectivity for the histamine receptor is unaltered by this variation, the E isomer of (27) is 11 times more potent than zimeldine at guinea pig ileum sites (this isomer is, however, a relatively feeble antihistaminic - 15 to 20 times less active than brompheniramine). Zimeldine (27) is used clinically as an antidepressant agent.



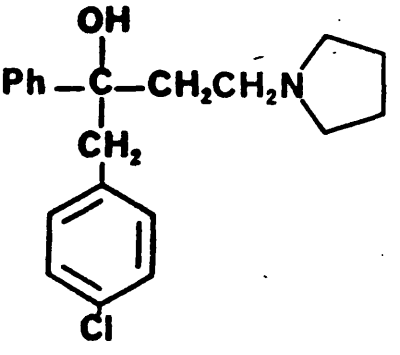
(27)

1.4.2 1,2-diaryl-4-aminobutenes

The clinically important member of this group is pyrrobutamine (28) diphosphate, which is one of the four dehydration products produced from the tertiary alcohol (29).



(28)



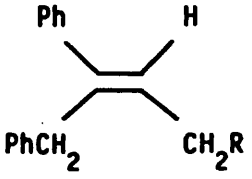
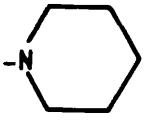
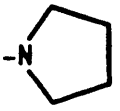
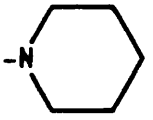
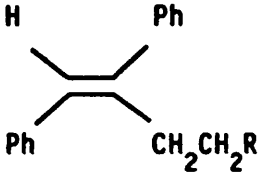
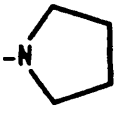
(29)

A problem arises with respect to the structure of butenes derived from this alcohol (29) - since it may lose water in either two directions giving but-1-ene or but-2-ene mixtures, each of which may exist as an E/Z mixture.

The position of the double bond and the configuration of pyrrobutamine remained doubtful for some years. The accurate characterization was made possible by the application of ^{1}H-NMR and UV spectroscopy (Casy and Ison, 1970) and established it as E-but-2-ene (28). Pyrrobutamine (28) disphcsphate is one of the most potent antihistamines known (log K_b 10.34 at equilibrium guinea pig ileum sites) and significantly more active than its Z-but-2-ene and but-1-ene analogues (Ison et al, 1973). The but-1-enes are still, however, reasonably potent antihistaminic agents with log K_b values of 8.12 (E) and 8.65 (Z isomer).

The pyrrolidino basic group of pyrrobutamine and its isomers makes an important contribution to the potency as shown in Table 1.9 (Ison et al, 1973) as compared with the previously discussed propenes.

Table 1.9 : Potency data for some analogues of pyrobutamine

Isomer	R	Log K_b
But-2-enes		
	$-NMe_2$	8.45
		8.70
		9.64
But-1-ene		
		7.50
		8.16

1.5 Sedation and Antihistamines (Reinhardt and Borchard, 1982; Nicholson, 1983; Levander et al, 1985)

Sedation is the most prominent side effect of antihistamines, although the extent may vary from patient to patient as much as with drug to drug. The question of mechanism and especially whether these side effects are due to blockade of histamine receptors in the central nervous system is still unclear. Uzan et al (1979) reported that mequitazine (a phenothiazine derivative with a low level of side effects) showed less binding for central H_1 receptors and therefore suggested that the sedative effects of antihistamines may be associated with H_1 receptor blockade

The majority of 'older' H_1 receptor antagonists appear to display sedative effects because of their ability to cross the blood-brain barrier. Two recently introduced antihistamines i.e. terfenadine and astemizole appear to be non-sedating (Nicholson and Stone, 1982) due to their reduced ability to cross the blood-brain barrier.

There have been many studies to assess the sedative effects of a number of antihistamines in humans. The method involves comparison of a placebo with at least two antihistaminic agents e.g. terfenadine / chlorpheniramine (Kulrestha et al, 1978); brompheniramine / triprolidine (Nicholson, 1979); triprolidine / clemastine (Peck et al, 1978); hydroxyzine / clemastine / azatadine (Levander et al, 1985). Assessment of the side effects involves a number of performance tests and evaluation of subjective drowsiness. The reported data clearly indicates the

drug to drug variation of relative histaminic blockade and sedation activities.

A reduction of these drug related sedative effects has already been achieved by chemical modification of the H_1 antagonist structure i.e. terfenadine, astemizole and cetirizine. The reduced ability to cross the blood-brain barrier is due to metabolic changes in their structure (e.g. formation of COOH group) rendering them more hydrophilic. In the case of racemic antihistamines it may be possible that administration of one enantiomer may combine active antihistaminergic properties with the absence of sedation. On the other hand, the active H_1 antagonistic enantiomer may also induce sedation while the less active enantiomer does not. A result of this kind would lend support to the view that sedation is linked to histamine stimulation of central H_1 receptors. A study of this kind, in which individual isomers of enantiomeric pairs will be administered in an *in vivo* study and any central sedative effects quantified, form one of the aims of this thesis.

1.6 Aims and Objectives of the Work

The aims and objectives of this work are to re-evaluate and extend the knowledge of potency differences between isomeric pairs of H_1 antihistaminic agents with special reference to the following points:

- The study of differences in pharmacological potency of enantiomeric pairs at both central and peripheral receptor sites by *in vitro* (guinea pig ileum assay and binding studies) and *in vivo* methods (in animals and possibly humans).
- The absolute configurational requirements of H_1 antagonists at the receptor site, correlating the data for existing compounds and also evaluating data of novel cases.
- To study semi-rigid antihistamines of the aminopropene type (e.g. triprolidine). To identify configurational requirements and correlate these with the pheniramines and related chiral agents e.g diphenhydramines. To attempt additional S.A.R. studies by the sythesis of novel amino-propene type antihistamines with differing pyridyl substituents e.g. replacing the 2-pyridyl with 3- or 4-pyridyl and/or varying the aryl substituent.

In order to carry out this work it was necessary to resolve a variety of chiral antihistamines on a scale that provided enough material for a range of pharmacological assays including binding studies and *in vivo* work. In previous work on isomers of this kind no special attempts were made to establish the isomeric purity of the materials examined. It was important, therefore, to firstly examine critically the method usually employed for the assessment of optical purity i.e. optical rotation. Since this method is not an 'absolute' method it led to the study of chiral analysis by ^1H NMR and HPLC.

Chapter 2

Chiral Resolution by Fractional Crystallization

2.1 Introduction

A chiral molecule is one which contains an element of dissymmetry such that the molecule may exist in two forms related as object to mirror image. A commonly encountered dissymmetric element is an asymmetric carbon atom i.e. a carbon atom with four different substituents attached (Fig 2.1).

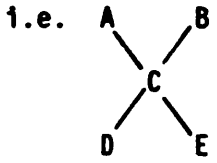


Fig 2.1

A chiral molecule exists in two forms which bear an object-mirror image relationship and are termed enantiomers, enantiomorphs or optical antipodes. These isomers are optically active in the sense that when a beam of plane-polarized light passes through a solution of one enantiomer, the plane of polarization is rotated. Moreover, separate enantiomers rotate the plane of plane-polarized light in equal amounts but in opposite directions.

The number of degrees that the plane of polarization is rotated as it passes through a solution of an enantiomer depends on the number of chiral molecules that it encounters. This depends on the length of the polarimeter tube and the concentration of the enantiomer.

Specific rotation [α] is defined as the rotation produced by 1 g of substance in 1 ml of liquid in a 1 dm polarimeter tube.

Thus $[\alpha] = \frac{100\alpha}{c.l.}$

[α] = specific rotation

α = observed rotation

c = sample concentration (in g/100 ml)

l = polarimeter cell-length in decimetres

The specific rotation depends on:

(i) the temperature

(ii) the wavelength of the plane-polarized light

(iii) the solvent

(iv) the concentration.

Specific rotations are therefore reported to include these particulars.

Hence $[\alpha]_D^{25} = +33.7°$ (c. 1.0, MeOH)

means using the sodium D-line ($\lambda = 589$ nm), at a temperature of 25°C, the specific rotation of a sample containing 1 g per 100 ml of the optically active substance dissolved in methanol, in a sample tube of 1 decimetre length, produces a rotation of 33.7° in a clockwise direction.

The value of the observed rotation is measured with a polarimeter. This consists of a light source (historically a sodium lamp), a polarizing (Nicol) prism, a sample tube, an analyser prism with a circular scale and a detector (usually the eye). In principle the measurements can be made by first adjusting the two prisms to a crossed position to yield a minimum light intensity in the presence of pure solvent.

With an optically active sample in place, rotation of the beam causes an increase in light intensity which can be offset by rotation of the analyser prism. This angular change necessary to minimize the intensity again, corresponds to the rotatory power of the sample. Unfortunately the position of minimum intensity cannot be determined accurately with the eye and so polarimeters are equipped with a half-shadow device. This consists of a small Nicol prism that intercepts half of the beam emerging from the polarizer. Thus in the presence of pure solvent, with this Nicol prism at 90° with respect to the polarizer, a split light/dark field is observed. The light prism corresponds to that half of the beam that has been rotated by the small prism and the dark part corresponds to the unobstructed beam. In the presence of an optically active sample the analyser is rotated until the same balance is obtained, so that the two halves of the field are equally illuminated. A clockwise rotation corresponds to a dextrorotatory substance (α is positive) and an anti-clockwise rotation to a laevorotatory substance (α is negative).

The polarimeter used in the following experiments was an Optical Activity AA-10 polarimeter. Although the principle is the same as that employed in visually-balanced polarimeters, there are a number of factors which make this instrument especially useful and easy to use.

This polarimeter has a digital display which gives sample rotation directly in degrees. It also has a fan-cooled mercury arc lamp, (instead of a sodium lamp) coupled to a series of five interference filters mounted on a turret with means for wavelength 'tilt-tuning'. The light from the mercury arc is projected into the polarimeter where an adjustable plane mirror brings the beam into alignment with the optical path.

The five filters enable α values to be recorded at 589, 578, 546, 436 and 365 nm. The use of five wavelength measurements and not just one (589 nm) increases the validity of the results and enables greater scope for reproducibility. In general, the extent of rotation increases as the wavelength of observation decreases, hence sensitivity is enhanced by having access to wavelengths shorter than 589 nm.

Although specific rotational values aid the monitoring of resolution of chiral compounds it is not an absolute indication of optical purity. For instance, if (+)A is contaminated with a certain proportion of (-)A and vice versa, the rotation values will be identical except for sign. If recrystallization does not alter this proportion, one might assume two pure enantiomers are present. It is important, therefore, to develop methods where the proportions of each enantiomer can be visualised (Chapter 3).

Since the physical properties of enantiomers are identical, they cannot be separated by commonly employed methods e.g. not by fractional distillation, because their boiling points are identical, not by fractional crystallization, because their solubilities in a given solvent are identical (unless the solvent is optically active), not by chromatography, because they are held equally strongly on a given adsorbent (unless it is optically active).

The resolution of a racemic compound therefore requires a special kind of approach.

The most widely used method is conversion of a racemic mixture, by an optically active reagent, into a mixture of diastereomers which can, in principle, be separated because antipodal relationships no longer hold.

For example, a racemic base (±)B can be converted into a diastereomeric mixture of salts, $[(-)BH^+ (-)A^-]$ and $[(+)BH^+ (-)A^-]$, using enantiomerically pure acid (-)HA. These diastereomeric salts have non-identical physical properties including solubilities in a given solvent. They can therefore be separated by fractional crystallization. Once the two salts are separated, optically active base can be recovered from each salt by the addition of a stronger base (assuming there is no danger of racemization).

Naturally occurring optically active amine alkaloids such as (-)-quinine, (-)-strychnine and (-)-brucine are often employed as resolving agents for racemic acids using the same method as mentioned above.

Examples of the acids used for resolving racemic bases, such as those used in this work include (+)-L-natural or (-)-D-unnatural tartaric acids and their derivatives.

The assignment of the configuration of (+)tartaric acid (natural tartaric acid) has historically met with difficulties, because this acid has two asymmetric carbon centres. Prior to 1951, it was necessary to assign an arbitrary configuration to a molecule correlating it to another similar structure. In many cases (+)-glyceraldehyde was the compound of comparison, this arbitrarily being assigned the D- configuration.

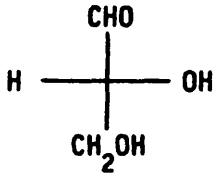


Fig 2.2 Fischer projection of (+)-glyceraldehyde.

In the case of tartaric acid, having two asymmetric centres, it is difficult to decide whether the natural (+)tartaric acid should be assigned the L-configuration using the bottom asymmetric carbon as the reference or D-tartaric acid using the top asymmetric centre as the reference.

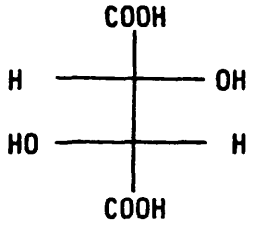


Fig 2.3 (+)Tartaric Acid

In 1951, Bijvoet et al (Bijvoet et al, 1951) successfully analysed the sodium rubidium salt of natural tartaric acid by X-ray crystallography to show it as the L-configuration i.e. the hydroxyl group is on the left hand side of the lower asymmetric carbon atom.

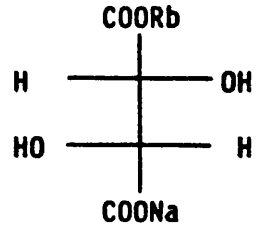


Fig 2.4 Sodium rubidium salt of (+)tartaric acid

Tartaric acid is a clear example where an unambiguous system for the specification of absolute configuration is necessary. In 1956, Cahn, Ingold and Prelog (Cahn, Ingold and Prelog, 1956) proposed such a system. Taking each asymmetric centre in turn, the four groups attached to the asymmetric carbon are arranged in a priority sequence, based on decreasing atomic number and other criteria. The molecule is then viewed with the group of lowest priority pointing away from the viewer. If the remaining three groups trace a clockwise priority order, the configuration about that carbon is designated as R (rectus), if anti-clockwise, by S (sinister).

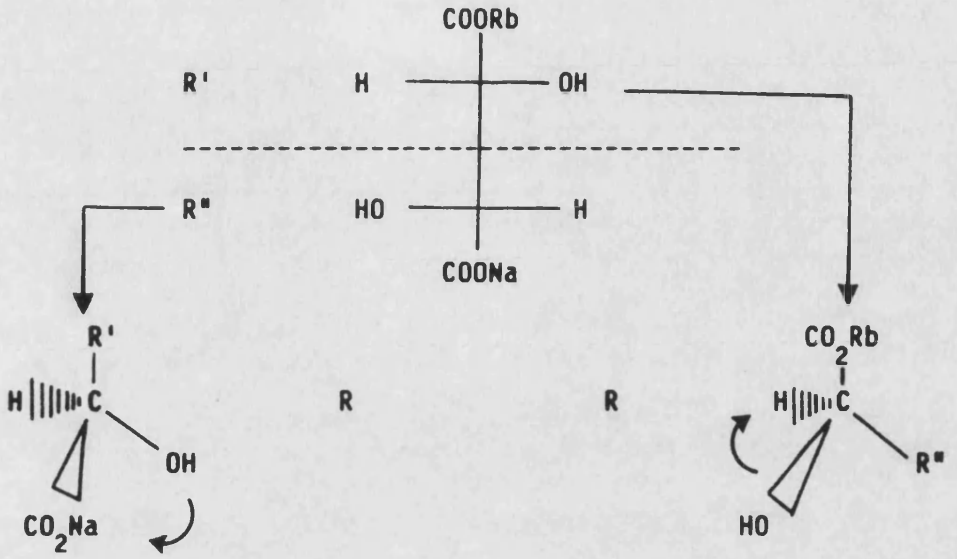


Fig 2.5

For the tartaric acid salt (Fig 2.5), looking at the lower asymmetric carbon, the priority order, with the hydrogen atom pointing away, will be OH> CO_2Na > R' i.e. R configuration. Similarly at the second asymmetric centre, the same pattern is observed i.e. the R configuration. Therefore a non-ambiguous way to describe the configuration of L-(+)tartaric acid is R,R,(+)tartaric acid.

2. Results and Discussion

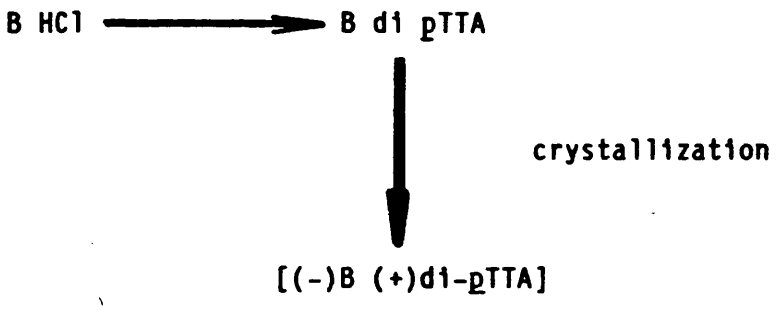
2.2 Chlorpheniramine

The resolution of chlorpheniramine (17c) was first reported by Roth & Govier (1958,) using phenylsuccinic acids as the resolving agents. The details (Scherico Patent, 1960) of the resolution involved the use of a molar equivalent of RS base and either (+) or (-) phenylsuccinic acid dissolved in ethanol. Resolution of phenylsuccinic acid (Wren and Williams, 1916) by fractional crystallization of the brucine salts from ethanolic solution was therefore undertaken, the (-)brucine salt of the (+)acid being the more sparingly soluble. After many weeks of purification, small samples of each of the antipodal phenylsuccinic acids were obtained (2.7.5).

Trials of the resolution of chlorpheniramine using the (+) and (-)phenylsuccinic acid proved unsatisfactory. After six months an oil but no crystalline solid had formed. Use of another optically active acid was deemed necessary.

References to the use of four other acids in the resolution of chlorpheniramine were found. These included tartranilic acid (Yoshitomi, 1975), p-nitro-tartranilic acid (Kongo Yakukin, 1976), N-tosylaspartic acid (Yoshitomi, 1973) and Di-p-toluoyltartaric acid (abbreviated to di-pTTA) (Hunt, 1961). For the first three acids listed above, no details were available and thus di-pTTA was the next trial acid to be employed.

Brittain, D'Arcy and Hunt (1959) reported the resolution of chlorpheniramine using di-pTTA in ethanol but stated that only after 1 year was any crystallization seen. Our attempts using RS chlorpheniramine:di-pTTA (1:1 ratio) yielded only a green oil. Hunt (1961), reported an improvement of this method "by the substitution of HCl for half an equivalent of the active acid". In this way an equilibrium was established between the salts of RS base(B) - HCl and (+) or (-)base with the di-pTTA. When the less soluble diastereomeric salt crystallizes this shifts the equilibrium to the right, thus increasing the formation of the di-p-toluoyltartrate salt.



Communication with Dr Hunt (now sadly deceased) indicated an 18 month time lag was necessary before any crystallization occurred. Fortunately some seed crystals were available and kindly donated, and their use successfully induced the first crystallization which was complete within one month.

Initially the two enantiomers of chlorpheniramine were obtained by treating RS chlorpheniramine with either (+) or (-)di-pTTA. Since, however, the laevorotatory di-p-toluoyl derivative of natural L-(+)tartaric acid is more readily available (and less expensive) further resolutions involved recovery of the (-)base from the mother liquors of the reaction of RS chlorpheniramine and (-)di-pTTA, these being enriched in the (-)isomer of chlorpheniramine. The (-)isomer was then treated with the dextrorotatory di-pTTA, obtained from unnatural D-(-)-tartaric acid. Recrystallization of both of these diastereomeric salts was continued until a constant rotation value was obtained and by comparison to literature values (Hunt, 1961). The salt obtained using (-)di-pTTA had an overall (-)rotation ($[\alpha]_D^{25}$ - 60.7, c. 1.19, 95% EtOH) which on basification gave (+)chlorpheniramine base ($[\alpha]_D^{25}$ + 25.6, c. 1.44, 95% EtOH).

The two antipodal bases of chlorpheniramine were converted to their maleate salts for pharmacological testing (Chapter 6). The maleic acid had no effect on the sign of rotation i.e. (+)chlorpheniramine base as a maleate salt carried a (+)rotation in water and DMF ($[\alpha]_D^{25}$ + 23.3, c. 1.2, H_2O and $[\alpha]_D^{25}$ + 41.1, c. 1.0, DMF). Comparison of these maleate rotation results to the literature indicates a good correlation between the di-p-toluoyltartaric acid (Hunt) and phenylsuccinic acid (Scherico) methods.

2.3 Dimethindene

The resolution of dimethindene (20) had been reported (Huebner, et al, 1960) using tartaric acid as the resolving agent, but no details were included. Our attempts using a molar equivalent of tartaric acid to dimethindene base in ethanolic solution led to crystalline salts and the first recrystallization was complete in 24 hours. After four such recrystallizations from absolute ethanol a constant rotation value had been reached. These diastereomeric salts were suitable for pharmacological testing since the tartrate anion is inert in this sense (Chapter 6).

Again it was the (-)base that gave the less soluble diastereomeric salt with the (+)acid - although in this case the salt had an overall (-)rotation ($[\alpha]_D^{25}$ - 146.1, c. 1.07, methanol). The derived oily base had a high negative rotation ($[\alpha]_D^{25}$ - 144, c. 1.01, methanol) sufficient to counteract the positive contribution from the L-(+)-tartaric acid ($[\alpha]_D^{20}$ + 12.0, c. 20, water).

Conversion of this salt to its maleate derivative followed, for direct comparison with the marketed racemic maleate (Fenostil,Zyma). The salt of (-)-dimethindene and maleic acid, as expected had a (-)rotation, although higher than that of the original (-)base. ($[\alpha]_D^{24}$ - 206.6, c. 1.00, methanol). Similarly (+)dimethindene maleate had a rotation value of the same order i.e. $[\alpha]_D^{25}$ + 197.8 (c. 1.02, methanol).

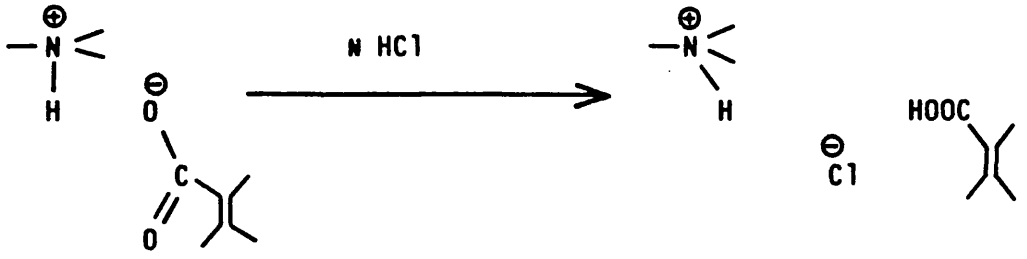
2.4 Carbinoxamine

Resolution of carbinoxamine (9) has been reported without details (Roszkowski and Govier, 1959) using tartaric acid as the resolving agent in ethanolic solution. After six months of trials at Bath, using a variety of solvent mixtures e.g. methanol, ethanol, ethanol/ethyl acetate - only oily residues formed. Some seed crystals were then obtained from McNeill Laboratories. Seeding an absolute ethanol mixture of L(+)-tartaric acid and RS carbinoxamine base, with carbinoxamine-L-tartrate and leaving at room temperature for two weeks successfully induced crystallization. Collection and recrystallization followed to yield the diastereomeric salts. Again the least soluble salt was that of (-)base (+)acid. The resolved carbinoxamine bases have very small rotation values i.e. $[\alpha]_D^{25}$ + 6 and -8 respectively and therefore their tartrate salts take on the sign of the acid used i.e. the salt of (-)carbinoxamine and (+)tartaric acid had a constant rotation $[\alpha]_D^{25}$ + 45.8 (c. 1.07, MeOH), the other salt having an $[\alpha]_D^{25}$ - 46.0 (c. 1.00, MeOH).

Carbinoxamine does not follow the same pattern as the two previous examples after conversion to the maleate. When the maleate salt of carbinoxamine was made the sign of rotation was opposite that of the base used. Thus the maleate salt, formed from (-)carbinoxamine base, $[\alpha]_D^{25}$ - 8.0 (c. 0.96, absolute ethanol) and maleic acid, had a rotation $[\alpha]_D^{25}$ + 40.8 (c. 1.01, MeOH). Similarly (+)carbinoxamine base, $[\alpha]_D^{25}$ + 6.0 (c. 1.00, abs EtOH) formed a maleate salt with $[\alpha]_D^{25}$ - 47.8 (c. 0.98 in MeOH).

Although the maleates were found to differ in the magnitude of their rotation $[\alpha]_D^{25}$ + 40.8 and - 47.8 (in methanol) respectively, such a difference was not seen in the tartrate salts, these two salts having similar magnitudes of constant rotation in methanol of + 45.8 and - 46.0 respectively. It is unlikely that any racemization has occurred during the transfer of tartrate to maleate salt. Another possible explanation was the association of differing quantities of solvent of crystallization with the two diastereomeric salts. This possibility was eliminated by micro-analysis results which show no solvent of crystallization to be present (2.7.3).

It would appear, therefore, that the difference in magnitude of the two maleate diastereomers is due to the maleic acid. The rotation value of another salt was necessary for comparison. The new salt chosen was that of a hydrochloride. Addition of a few drops of N-HCl to a polarimeter cell containing the respective maleate salt, changed the rotation dramatically.



	$[\alpha]^{25}$ Carbinoxamine Maleate			
nm	(+) Base		(-) Base	
	MeOH	MeOH + HCl	MeOH	MeOH + HCl
589	- 47.8	- 135.2	+ 40.8	+ 130.2
578	- 48.8	- 140.2	+ 44.7	+ 138.2
546	- 56.9	- 163.6	+ 49.7	+ 160.0
436	- 102.6	- 320.1	+ 97.4	+ 314.1
365	- 189.0	-	+ 182.0	-

Table 2.1 The change in rotation of carbinoxamine maleates on addition of N HCl.

Changing the salt, from maleate to hydrochloride has a drastic effect on the rotation. As hydrochlorides the differences in the rotations was only ≃ 3% whereas as maleates a 14% difference in the two values was seen. This suggests that the maleate counter ion differentially influences the rotational magnitudes of the optically active (-) and (+) cations. A similar phenomenon was observed in regard to the ^{1}H NMR features of the maleate salt of dimethindene (4.4.3) when compared to those of tartrate and fumarate salts.

2.5 Mebrophenhydramine

The resolution of mebrophenhydramine (11) has not previously been reported. Trials using tartaric acid, di-pTTA, d-10-camphorsulphonic acid, as 1:1 molar equivalents were therefore carried out. Only an oily product was formed with tartaric acid (95% EtOH) and di-pTTA (95% EtOH) and there was no success either with d-10 CSA (95% EtOH). The use of di-pTTA (abs EtOH) proved more successful and after four weeks at room temperature the crystals which formed were collected.

The crystals collected from reaction of RS base with (-)di-pTTA, having an overall (-)rotation $[\alpha]_D^{25}$ - 89.4 (c. 0.928, MeOH) were in fact the salt formed between (-)mebrophenhydramine base $[\alpha]_D^{25}$ - 32.1 (c. 5.02, MeOH) and (-)di-pTTA - the opposite of the three previous examples. Recovery of the (+)antipode enriched base from the mother liquors was followed by formation of (+)di-p-toluoyl tartrate $[\alpha]_D^{25}$ + 91.2 (c. 0.95, MeOH). As no literature values were available it was essential to ensure constant rotation had been reached.

Formation of the maleate salt, highlighted the effect of the maleate counter ion, on this type of structure. Again the two maleates had very different [α] values. The salt of (-)mebrophenhydramine and maleic acid had a rotation value of $[\alpha]_D^{25}$ - 20.9 (c. 1.00, MeOH) and similarly the maleate salt of the (+)base had a rotation value of $[\alpha]_D^{25}$ + 5.06 (c. 1.0, MeOH).

In this case the difference is even more pronounced than that seen for the antipodal maleates of carbinoxamine. As di-pTTA salts the rotation for the two diastereomers are closely comparable, results which suggest that resolution is complete. As maleates, however, one would question that the two salts are fully resolved. As in the case of carbinoxamine racemization or the presence of solvent of crystallization (from micro-analysis results - 2.7.4) can be eliminated as an explanation for this phenomenon.

Again, addition of N-HCl, to the respective maleates, to form hydrochlorides, alters the rotation although not as much as in the previous case.

$[\alpha]^{25}$ mebrophenhydramine maleate

(nm)	(+)base	
	MeOH	MeOH + HCl
589	+ 5.06	+ 8.10
578	+ 7.08	+ 9.11
546	+ 8.10	+10.12
436	+18.22	+19.23
365	+30.36	-

These results emphasize the need for measurement of optical purity by absolute methods, which are less ambiguous and more reliable - see later discussions using circular dichroism (CD), HPLC and ^{1}H NMR.

Other attempted resolutions included that of RS doxylamine with d-10 CSA (EtOH, MeOH) and di-pTTA (EtOH) but no crystalline solid formed.

In addition, the resolution of meclozine and hydroxyzine was undertaken. These compounds, having two basic nitrogens, required a 1:2 molar ratio of base:acid. Trials using d-pTTA (EtOH), d-10 CSA (EtOH) and tartaric acid (95% EtOH, abs EtOH) were set up. After 12 months a crystalline solid began to form with tartaric acid (abs EtOH). This was collected but unfortunately there was insufficient time for further recrystallizations to be completed.

2.6 Circular Dichroism (CD)

2.6.1 Introduction

Circular dichroism (CD), like optical rotary dispersion (ORD), is an aspect of the feature of optical activity known as the Cotton effect. It is a consequence of the fact that when a beam of plane polarized light (PPL) is passed through a solution of an optically active substance which also exhibits specific absorption, in the region of the absorption band the two circular polarized beams, into which the incident plane polarized beam is split, are absorbed to different extents. This gives rise to a rotated elliptically polarized beam, the origin of which is readily visualized in Fig. 2.6.

Plane polarized light may be regarded as made up of right and left handed circularly polarized light. The two components travel through an optically active medium at different speeds (producing rotation of the plane of polarized light) and suffer different degrees of absorption if the medium includes an appropriate chromophore. The rotation α is the angle between the major axis of the ellipse and the plane of the incident beam. The ellipticity is defined as the angle (ψ) whose tangent is given by the ratio of minor and major axes. CD is the result of this differential absorption and can be measured in terms either of the absorption difference or ellipticity to which it gives rise.(Crabbe, 1965 ; Drake, 1986[1] ; Velluz et al, 1965). Historically CD was detected through this change in polarisation (linear to elliptical).

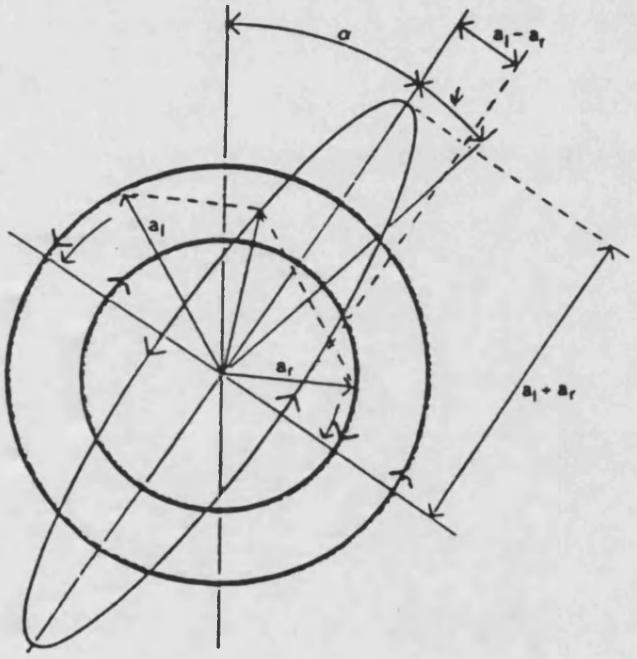


Fig 2.6 Basis of elliptical polarization and circular dichroism

Plane of incident polarization is rotated to the right or left according to whether the left circular vector rotates slower or faster than the right circular vector. If there is differential absorption of the two beams then the resultant amplitude vector will trace out an ellipse.

Modern instrumentation however is able to effectively generate individual alternate pulses of left and right circularly polarized light using an electro-optic modulator. The polarisation modulated light beam passing through an optically active solution in the region of an absorption band falls onto a photomultiplier where it generates an out-of-balance signal that can be used to provide an accurate measure of differential absorption ($\Delta\varepsilon$).

CD spectra are presented as plots of wavelength against $\Delta\varepsilon$ where $\Delta\varepsilon$ is the differential molar absorptivity of left and right circularly polarized light,

$$\text{i.e. } \Delta\varepsilon = (\varepsilon_L - \varepsilon_R) = (A_L - A_R)\ c.\ l.$$

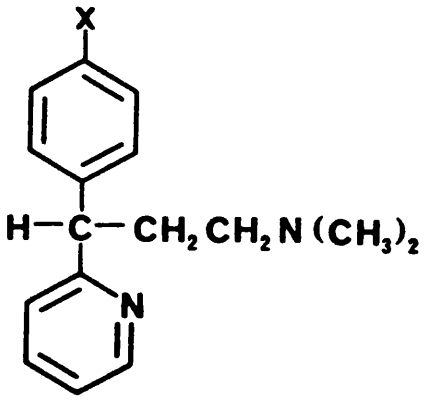
$\Delta\varepsilon$ is related to the molar ellipticity (ψ) (the traditional unit) and is either positive or negative. When positive i.e. $A_L > A_R$ it gives rise to a Cotton Effect band of positive sign and <u>vice versa</u>. The special stereochemical value of CD spectra rests on the fact that the sign of the CD band is related to the absolute configuration of a chiral centre close to the UV chromophore.

Thus a group of optical antipodes of related chirality and with similar chromophores give rise to CD effects of identical sign. This is the basis of the use of this technique for configurational assignments. (Crabbe, 1965 ; Drake, 1986[2]). To employ this technique, chiral centres of similar chemical structure must be present in the test compounds.

2.6.2 Results and Discussion

All the molecules analysed by this method contain aromatic chromophores close to the chiral centres.

2.6.3 Pheniramine type

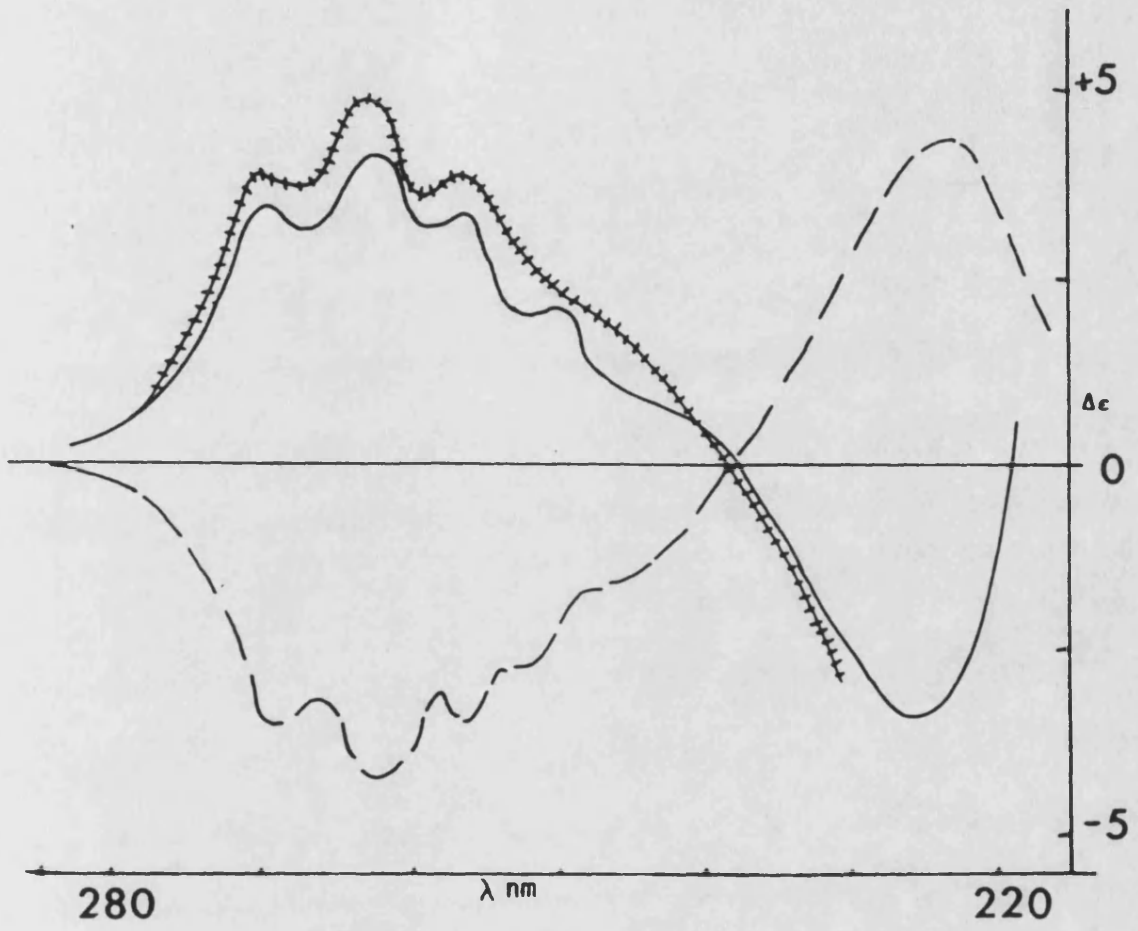


X = H (17)

X = Br (17b)

X = Cl (17c)

The CD spectra of (+) and (-)chlorpheniramine (17c) are related as object to mirror image (Fig 2.7). That of (+)brompheniramine (17b) closely resembled that of (+)chlorpheniramine, both known to be the more active antipodes of S configuration (Shafi'ee and Hite 1969), in both sign and fine structure as shown by Fig 2.7. These results emphasize the closely related CD features of pheniramine derivatives of identical configuration and the object to mirror image relationship of R-CD and S-CD curves.



(+) chlorpheniramine maleate

(-) chlorpheniramine maleate

(+) brompheniramine maleate

Fig 2.7a CD spectra for some pheniramine isomers in H_2O.

The CD features of both (-) chlorpheniramine and (+) brompheniramine were sensitive to pH (Fig 2.7b)

	pH	λ max/min	Δε
(-) chlorpheniramine	4.7-6.1	264	-0.43
	2.2	265	+0.74
(+) brompheniramine	5.3, 6.8	264	+0.48
	3.4	266	-0.53

The sign of the CD band is progressively reversed when a rise in the population of diprotonated species occurs (pyridyl nitrogen will only protonate at low pH values).

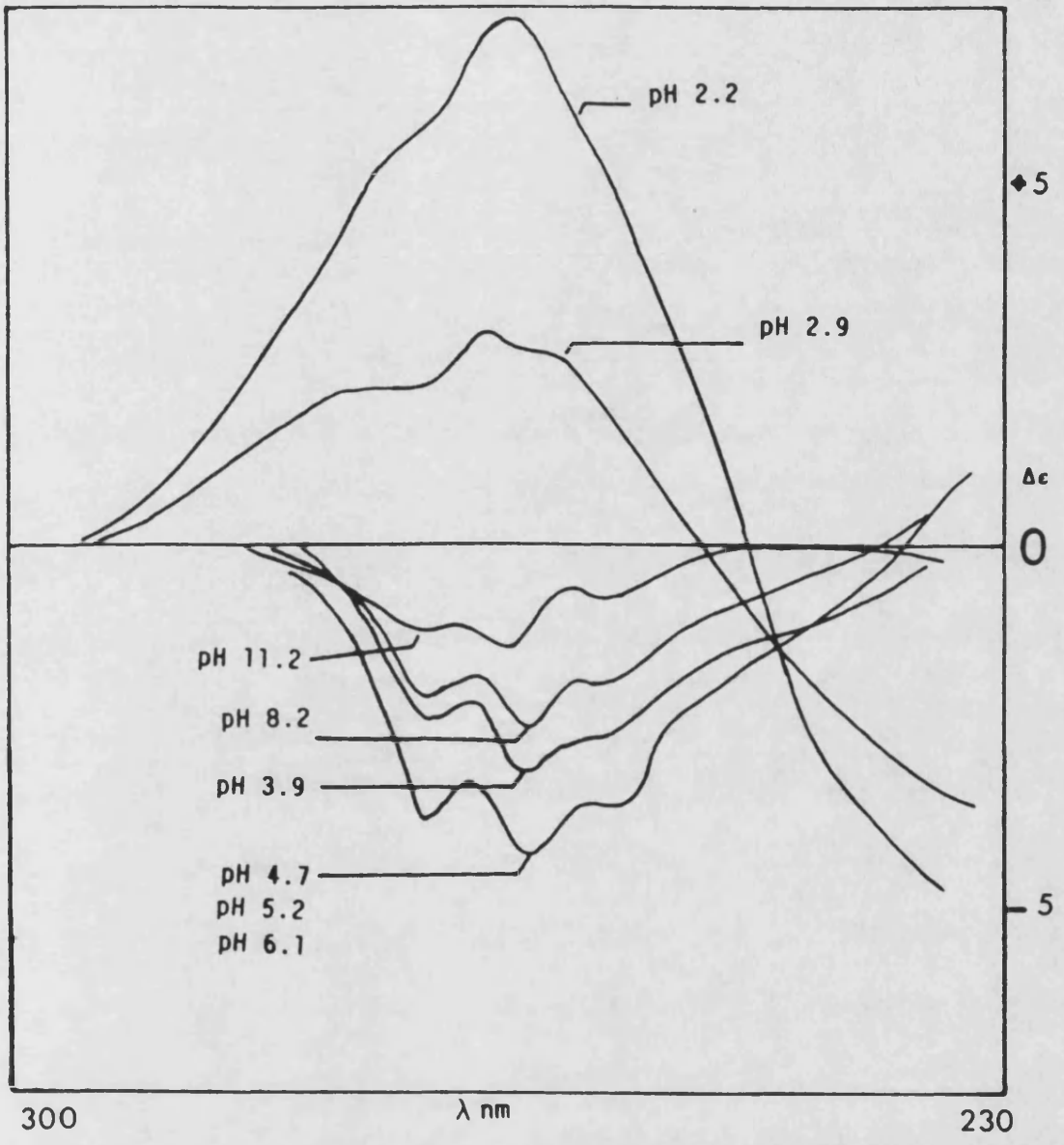
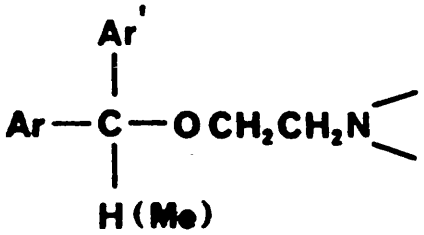


Fig 2.7b CD spectra for (-) chlorpheniramine showing the effect of pH on the sign of the band

2.6.4 Diphenhydramine type



In the region of 240-270 nm, the CD features of (+)neobenodine HCl (8) (the more potent antipode) resemble those of (+)chlorpheniramine and (+)brompheniramine - although at lower wavelength the pattern is different (Fig 2.8). This is evidence that (+)neobenodine is related in configuration to the (+)pheniramines a relationship established on chemical grounds (Barough et al, 1971).

The CD features of antipodal forms of carbinoxamine (9) show a broad maximum in the region of 230-280 nm. Both (-) carbinoxamine and (+) neobenodine show maxima of positive sign in this region in accord with their chemically related configuration. The fact that their maxima differ in fine structure is not unexpected in view of the fact the neobenodine contains two homoaryl sustituents while carbinoxamine is a homo/heteroaryl derivative.

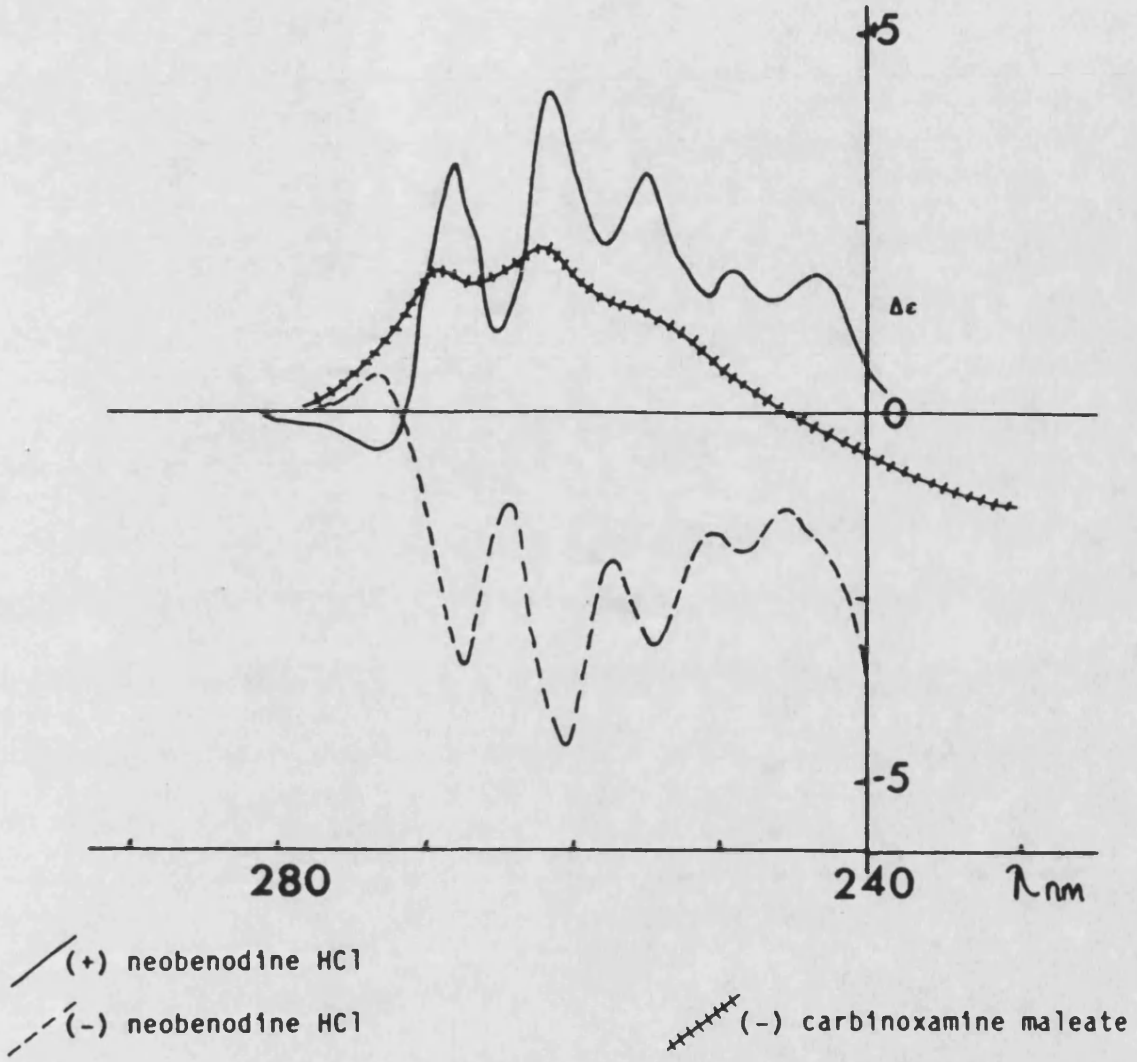
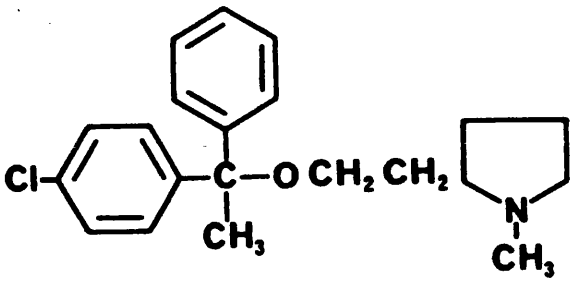


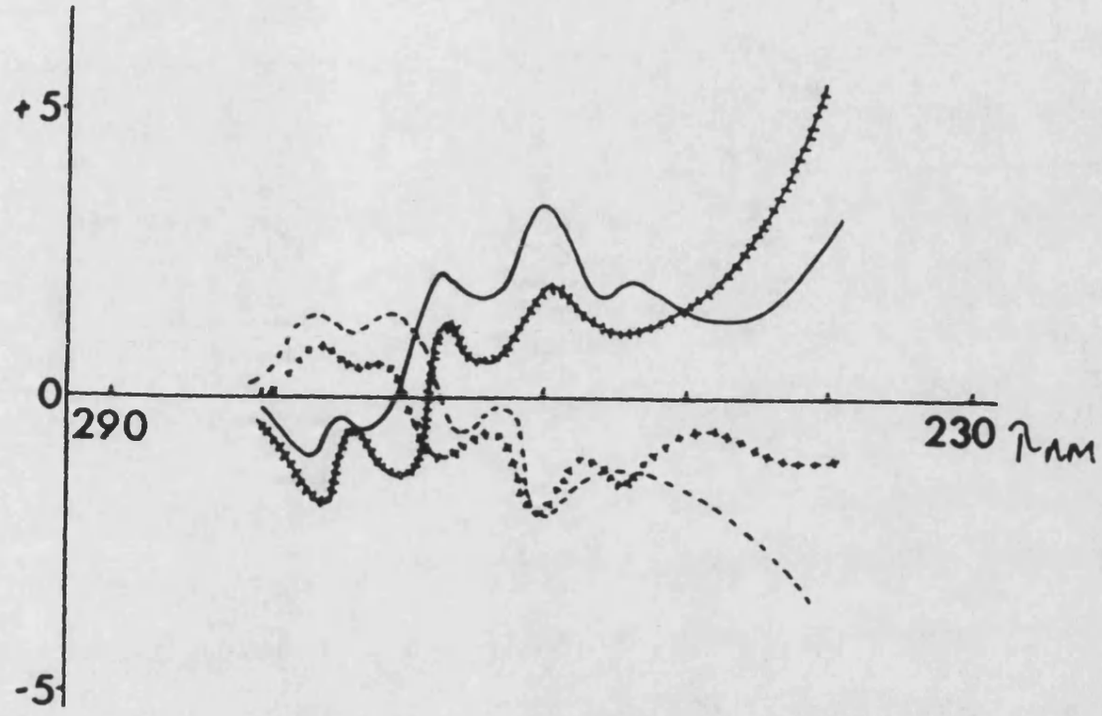
Fig 2.8 CD spectra for (+) and (-) neobenodine hydrochloride and (-) carbinoxamine maleate in H_2O

Clemastine (12) is another example of this type of compound, although it has two chiral centres, one at the benzylic site and the other in the pyrrolidino ring.



(12)

The four possible isomers are all of known configuration i.e. RR', RS', SR' and SS' (Ebnother and Weber, 1976). Study of the CD characteristics of these isomers is illustrated in Fig 2.9.



RR' ——— RS' +++++

SS' * * * * SR' - - - -

Fig 2.9 CD spectra for clemastine fumarate isomers in water

Fig 2.9 shows that the isomers RR' and RS' have similar CD features as do SS' and SR' but that the latter are mirror images of the former. This indicates that the CD bands are little influenced by the chirality of the pyrrolidino chiral centre possibly because this is too far removed from the chromophore.

The CD features of the RR and SS isomers are remarkably similar to those of (+) and (-) neobenodine respectively and the data provides further proof of the configurational assignments made to these enantiomers. The CD features of chiral molecules of this kind are not significantly altered when hydrogen attached to the chiral centre (as in (17) and (8)) is replaced by methyl (as in (12)).

Clemastine is a good model for mebrophenhydramine (11) a compound for which no evidence of the antipodal configuration has been reported.

The fact that the CD features of the most active (-)antipode of mebrophenhydramine correspond clearly with those of clemastine (RR') and its RS' diastereomer, provide good evidence of the trio having benzylic chiral centres of identical (R) configuration. (see Fig 2.10).

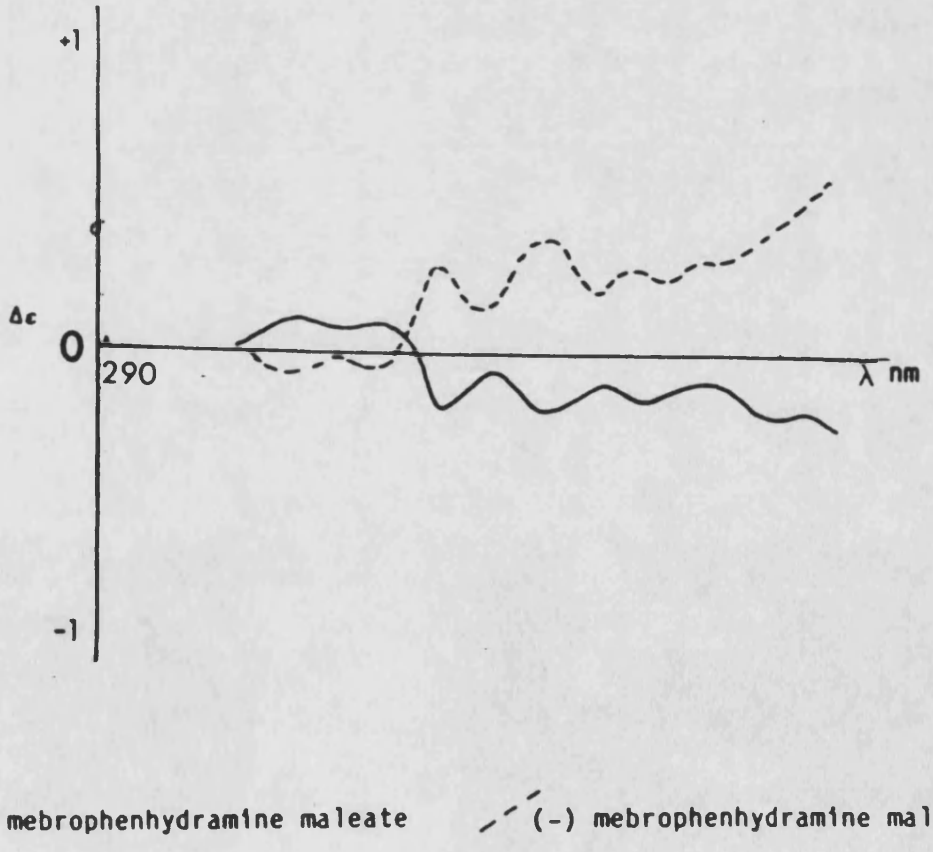
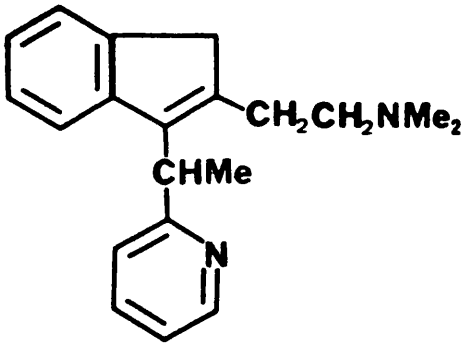


Fig 2.10 CD spectra for (+) and (-) isomers of mebrophenhydramine maleate in H_2O

2.6.5 Dimethindene Maleate



(20)

At pH 3.0, 3.5 and 4.3, three bands are seen :

positive band max, 275nm
negative band max, 250nm
positive band max, 245nm

$\Delta\varepsilon$ +0.07

At pH 5.8, 5.5, one band is visible

negative band min, 260nm $\Delta\varepsilon$ -0.68

At pH 8.5 $\Delta\varepsilon$ -0.8

These data again demonstrate the influence of the protonation state of the molecule upon its CD characteristics. The signs of the CD bands cannot be used as configurational evidence because the chiral centre of dimethindene differs radically from the other compounds examined in these experiments.

2.7 Experimental Details

2.7.1 Resolution of RS Chlorpheniramine by Di-p-toluoyl tartaric acids

RS chlorpheniramine base (liberated from RS chlorpheniramine maleate) (24 g, 0.09 M) and (-)-di-p-toluoyl-L-tartaric acid monohydrate (16.7 g, 0.041 M) were dissolved in warm ethanol (60 ml), and water (80 ml) containing N HCl (43.1 ml, 0.043 mol) was added. The solution was cooled slowly until clouding commenced, then seeded with a sample of the di-p-toluoyl L-tartrate provided by Dr J Hunt and allowed to cool overnight to room temperature. After 1 week the crystals which formed were filtered, washed with 30% aqueous EtOH (50 ml) and dried. Three recrystallizations from 50% aqueous EtOH gave an L-p-toluoyl tartrate of constant rotation, mp 135°C $[\alpha]_D^{25}$ - 60.7 (c. 1.19, 95% aq. EtOH) (Hunt (1961) $[\alpha]_D^{20}$ - 57.8 (c. 1.7, EtOH).)

Evidence for constant rotation

Crystallization	$[\alpha]^{25}$ nm 589	578	546	436	365	WT (g)
1st crop	-55.3	-58.3	-70.2	-141.3	-286.6	21.85
1.	-56.4	-60.3	-69.1	-142.0	-287.9	17.32
2.	-60.8	-64.4	-74.2	-156.5	-316.6	13.62
3.	-60.7	-65.0	-74.2	-157.2	-319.8	12.30

The above salt (12.3 g) was suspended in water (44 ml) and basified with N-NaOH (44 ml). An ethereal extract of the base was dried ($MgSO_4$) and evaporated under reduced pressure to a yellow oil (+)chlorpheniramine base (4.7 g)

nm	589	578	546	436	365
$[\alpha]^{25}$	+25.6	+26.3	+31.9	+60.9	+129.5

c. 1,44 in 95% EtOH

Hunt (1959) $[\alpha]_D^{21.5}$ + 31.6 (c. 1.8, EtOH)

The maleate salt was prepared by adding a solution of maleic acid (1.86 g) in isopropyl acetate (34 ml) to a solution of the above base (4.4 g) in isopropyl acetate (10 ml). The salt, which crystallized on cooling, was recrystallized three times from isopropyl acetate to give (+)chlorpheniramine maleate, mp 113-115°, $[\alpha]_D^{24}$ + 23.2 (c. 1.2, H_2O).

Crystallisation	$[\alpha]^{24}$ nm					conc^n, solvent
	589	578	546	436	365	
1st crop	+20.5	+22.1	+22.9	+46.6	+95.7	1.22, H_2O
1.	+21.8	+23.8	+31.8	+52.6	+103.2	1.01, H_2O
	+39.0	+42.9	+49.5	+102.9	+223.8	1.05, DMF
2.	+23.2	+25.0	+26.8	+51.8	+103.6	1.12, H_2O
	+41.0	+43.8	+53.2	+103.5	+224.8	1.07, DMF
3.	+23.2	+25.2	+27.0	+51.6	+104.0	1.20, H_2O
	+41.1	+44.1	+57.1	+103.8	+225.3	1.00, DMF

Hunt, (1961) $[\alpha]_D^{24}$ + 23.1 (c. 1.2, H_2O)

Scherico Patent, (1960) $[\alpha]^{25}$ +44.3 (c. 1.00, DMF)

(Found: C, 61.49; H, 5.77; N, 7.21 calculated for $C_{20}H_{23}Cl\ N_2O_4$: C, 61.46;, H, 5.93; N, 7.17%)

In a similar way the RS base (fresh or enriched in one isomer) and (+)di-p-toluoyl-D-tartaric acid monohydrate gave the Di-p-toluoyl tartrate of constant rotation, mp 135°C, $[\alpha]_D^{25}$ + 62.3 (c. 1.01, 95% EtOH), [Hunt, (1961) $[\alpha]_D^{24}$ + 57.4° (c. 1.1, EtOH)].

Crystallization	$[\alpha]^{25}$ nm					conc^n in
	589	578	546	436	365	95% EtOH
1st crop	+62.7	+65.7	+74.5	+154.9	+312.7	1.02
2nd crop	+61.1	+65.1	+78.2	+152.3	+312.6	0.99
3rd crop	+62.3	+65.6	+79.1	+153.3	+312.8	1.01

By basifying the salt, (-)chlorpheniramine as an unpurified oil was obtained,

nm	589	578	546	436	365
$[\alpha]^{25}$	-19.8	-20.7	-24.0	-50.4	-106.6

c. 1.21 in 95% EtOH

Hunt, (1961) $[\alpha]_D^{21.5}$ - 31.6° (c. 1.8, EtOH)

The maleate salt, after two crystallizations had mp 112-114°C, $[\alpha]_D^{25}$ - 23.9 (c. 1.08, H_2O) or -42.6 (c. 1.03, DMF).

Crystallization	$[\alpha]^{25}$ nm					conc^{n}, solvent
	589	578	546	436	365	
1.	-22.4	-23.3	-26.1	-55.0	-107.3	1.07, H_2O
	-40.6	-42.5	-50.9	-107.6	-234.9	1.63, DMF
2.	-23.9	-24.9	-28.5	-57.1	-112.3	1.08, H_2O
	-42.6	-44.6	-54.3	-110.5	-238.4	1.03, DMF
3.	-24.1	-24.9	-28.7	-57.6	-113.4	1.04, H_2O
	-42.8	-44.7	-54.8	-113.2	-239.0	1.10, DMF

Hunt, (1961) $[\alpha[_{D}^{25}$ - 23.1 (c. 1.2, H_2O) and

Sherico Patent, (1960) $[\alpha]_{D}^{25}$ - 44.1 (c. 1.0, DMF).

2.7.2 Resolution of RS dimethindene by tartaric acids

RS dimethindene (14.8 g, 0.05 M) and L-(+)-tartaric acid (7.61 g, 0.05 M) were dissolved in warm absolute EtOH (30 ml). The solution was allowed to cool overnight to room temperature. The crystals which separated were filtered and following four recrystallizations from absolute EtOH gave the L-tartrate of constant rotation.

Crystallization	$[\alpha]^{25}$ nm 589	578	546	436	365	conc^{n} in MeOH
1.	-103.4	-108.2	-125.2	-239.1	-459.2	1.054
2.	-129.7	-137.1	-157.2	-302.7	-569.6	0.948
3.	-146.5	-154.5	-175.2	-330.7	-622.5	1.01
4.	-146.1	-153.6	-177.0	-334.3	-628.3	1.068

The above salt (3 g) was suspended in water (20 ml) and basified with NH_3-H_2O. The ethereal extract of the base was dried ($MgSO_4$) and evaporated to an oil, (-)dimethindene.

nm	589	578	546	436	365
$[\alpha]^{24}$	-144	-153	-172	-322	-581

c. 1.01 in MeOH

Basification of the mother liquors followed by ethereal extraction gave impure (+)dimethindene (7 g, 0.02 M) as a yellow oil. To this was added D-(-)-tartaric acid (3.6 g, 0.002 M) and the two warmed together in absolute EtOH. The crystals that formed on cooling were filtered and after four recrystallizations from absolute EtOH gave the D-tartrate salt to constant rotation.

Crystallization	$[\alpha]^{25}$ nm 589	578	546	436	365	conc^{n} in MeOH
1.	+132	+140	+162	+310	-	1.06
2.	+140	+149	+171	+328	+619	0.998
3.	+141	+150	+174	+331	+623	1.006
4.	+144	+155	+178	+338	+631	1.016

Suspending this salt (1.5 g) in water, basifying (NH_3) and extracting with ether gave (+)dimethindene base, as a yellow oil.

nm	589	578	546	436	365
$[\alpha]^{25}$	+136	+140	+164	+311	+583

c. 1.0 in MeOH

The (-)base (800 mg) was dissolved in EtOH, an equimolar quantity of maleic acid, in EtOH, added and the solution warmed. After cooling, the solution was flooded with anhydrous ether to yield (-)dimethindene maleate, mp 127-129°C,

nm	589	578	546	436	365
$[\alpha]^{24}$	-206.6	-214	-243	-456	-847

c. 1.00 in MeOH

(Found; C, 70.57; H, 6.91; N, 6.86; calculated for $C_{24}H_{28}N_2O_4$; C, 70.4; H, 6.50; N, 7.2%)

Similarly the (+)base gave (+)dimethindene hydrogen maleate, mp 127-129°C,

nm	589	578	546	436	365
$[\alpha]^{25}$	+197.8	+206.7	+240.2	+451.8	+839.6

c. 1.02 in MeOH

(Found; C, 70.55; H, 6.72; N, 6.39%)

2.7.3 Resolution of RS carbinoxamine by tartaric acids

RS carbinoxamine base, obtained by basification of the maleate salt (7.6 g, 0.026 M) and L-(+)-tartaric acid (3.92 g, 0.026 M) were dissolved in hot absolute EtOH (25 ml). The solution was allowed to cool slightly, seeded (with McNeill sample, rotoxamine-L-tartrate) and left for 14 days at room temperature. The crystals (4 g) were filtered, washed with anhydrous ether and recrystallized four times from absolute EtOH to give the L-tartrate salt of constant rotation, mp 142-143°C.

Crystallization	$[\alpha]^{25}$ nm 589	578	546	436	365	conc^{n} in MeOH
1.	+36.3	+36.3	+43.2	+73.7	+119.8	1.018
2.	+36.8	+40.0	+49.8	+84.4	+146.1	0.924
3.	+42.3	+44.2	+52.0	+89.4	+153.8	1.04
4.	+45.8	+45.8	+51.4	+87.9	+154.2	1.07

(McNeill value $[\alpha]_D^{26.5}$ + 38.4 (c. 2.00, MeOH)

(McNeill Patent (1962) $[\alpha]_D^{25}$ + 37.2 (c. 2.0, MeOH)

The tartrate salt (3 g) was dissolved in absolute EtOH and an excess of methylamine (in EtOH) added until the solution was basic to litmus. Removal of the solvent under reduced pressure yielded a white solid (methylamine (-)tartrate). This was washed with acetone and the suspension filtered. The acetone was removed under reduced pressure to give oily (-)carbinoxamine base (C.A., 58, 5643g $[\alpha]_D^{25}$ - 6.8 (c. 20, MeOH)).

nm	589	578	546	436
$[\alpha]^{25}$	-8	-10	-15	-33

c. 0.964, in Abs EtOH

The hydrogen maleate had mp 135-136°,

nm	589	578	546	436	365
$[\alpha]^{25}$	+40.8	+44.7	+49.7	+97.4	+182.0

c. 1.01 in MeOH

(McNeill Patent (1962) $[\alpha]_D^{25}$ + 41.2 (c. 2.0, MeOH))

(Found; C, 58.9; H, 5.6; N, 6.4. Calculated for $C_{20}H_{23}ClN_2O_5$; C, 59.04; H, 5.70; N, 6.89%).

The mother liquors from the formation of the L-tartrate salt were evaporated under reduced pressure, basified ($NaOH-H_2O$) and extracted with ether to yield a yellow oil (5.3 g). This was dissolved in warm EtOH and D-(-)-tartaric acid (2.6 g) added. After slight cooling this was seeded with carbinoxamine-D-tartrate (McNeill sample). After a few days the crystals which formed were collected. Three recrystallizations from absolute EtOH yielded the D-tartrate salt of constant rotation, mp 140-141°C.

Crystallization	$[\alpha]^{25}$ nm 589	578	546	436	365	conc^n in MeOH
1.	-40.3	-39.4	-44.2	-75.8	-127.6	1.04
2.	-44.5	-46.4	-52.4	-87.9	-150.2	1.01
3.	-46.0	-45.0	-52.0	-89.0	-152.0	1.00

(McNeill value $[\alpha]_D^{26}$ - 38.6 (c. 2.0, MeOH)

The D-tartrate salt (2.6 g) was dissolved in EtOH, made basic (methylamine) and extracted into acetone, as before, to yield (+)carbinoxamine base as a straw coloured oil.

nm	589	578	546	436
$[\alpha]^{25}$	+6.0	+7.0	+10.0	+26.0

c. 2.2 in Abs EtOH

It formed a hydrogen maleate, mp 135-137°C,

nm	589	578	546	436	365
$[\alpha]^{25}$	-47.8	-48.8	-56.9	-102.6	-189.0

c. 0.948 in MeOH

(Found; C, 58.95; H, 5.78; N, 6.79%)

2.7.4 Resolution of mebrophenhydramine by Di-p-toluoyltartaric acids

RS-mebrophenhydramine (4.1 g, 0.012 M) and (-)di-p-toluoyl -L-tartrate acid monohydrate (4.76 g, 0.012 M) were dissolved in warm absolute EtOH (20 ml). The solution was allowed to cool and left at room temperature for four weeks. The crystals which separated during this period were filtered, washed and recrystallized three times from absolute EtOH to give an L-di-p-toluoyltartrate of constant rotation.

Crystallization	$[\alpha]^{25}$ nm 589	578	546	436	365	conc^{n} in MeOH
1.	-77.9	-82.7	-94.1	-188.2	-378.3	1.05
2.	-82.9	-88.5	-101.9	-204.8	-408.7	1.04
3.	-89.4	-87.3	-102.4	-203.7	-405.2	0.928

These (600 mg) were dissolved in absolute EtOH, made basic with methylamine and the product isolated (as 2.7.3) to give (-)mebrophenhydramine,

nm	589	578	546	436
$[\alpha]^{25}$	-32.1	-34.3	-38.9	-74.3

c. 5.02 in MeOH

The oily (-)base was dissolved in absolute EtOH and an equimolar quantity of maleic acid added. Crystals readily formed on cooling of (-)mebrophenhydramine hydrogen maleate (300 mg), mp 144-146°C

nm	589	578	546	436	365
$[\alpha]^{25}$	-20.9	-17.9	-19.9	-30.9	-50.8

c. 1.00 in MeOH

(Found; C, 56.97; H, 5.56; N, 3.13, calculated for $C_{22}H_{26}$ Br NO_5; C, 56.91; H, 5.64; N, 3.02%)

The other isomer, (3.91 g) impure, enriched in the dextro antipode was collected, as previously described, by basification of the mother liquors. Addition of (+) di-p-toluoyl-D-tartaric acid (4.54 g) in warm EtOH, followed by three recrystallizations from absolute EtOH gave a Di-p-toluoyl tartrate (630 mg) salt of constant rotation,

Crystallization	$[\alpha]^{25}$ nm 589	578	546	436	365	conc^n in MeOH
1.	+78.3	+85.1	+99.6	+200.2	+398.4	1.03
2.	+80.2	+86.1	+102.0	+205.0	+405.0	1.01
3.	+90.8	+98.2	+111.7	+224.3	+429.5	1.01
4.	+91.2	+97.5	+112.2	+221.2	+431.9	0.954

Basification of the salt, gave oily (+)mebrophenhydramine base (430 mg).

nm	589	578	546	436	365
$[\alpha]^{25}$	+28	+30	+34	+61	+109

c. 1.4 in MeOH

and formation of hydrogen maleate followed to give (+)mebrophenhydramine hydrogen maleate (225 mg), mp 140-142°C,

nm	589	578	546	436	365
$[\alpha]^{25}$	+5.06	+7.08	+8.10	+18.22	+30.36

c. 0.988 in MeOH

(Found; C, 56.88; H, 5.40; N, 2.92%)

2.7.5 Resolution of RS phenylsuccinic acid by brucine

RS phenylsuccinic acid (6.15 g, 0.03 M) and (-)brucine.$2H_2O$ (27.3 g, 0.06 M) were dissolved in warm 90% EtOH (490 ml). After 4 days the crystals that formed were filtered, washed, dried and recrystallized twice from 90% EtOH to form a (-)brucine salt of (+)phenylsuccinic acid of constant rotation,

	$[\alpha]^{25}$ nm					
Crystallization	589	578	546	436	365	conc^n in 95% EtOH
1.	-13.5	-14.5	-19.4	-54.6	-132.8	1.032
2.	-13.6	-13.6	-17.5	-53.5	-129.4	1.028
3.	-14.1	-14.1	-20.2	-54.4	-137.1	0.992

The above salt (12.0 g) was dissolved in water, made acidic (c. HCl) and extracted with ether. Evaporation of the ether under reduced pressure followed by four recrystallizations from water yielded (+)-phenylsuccinic acid (2.48 g) constant rotation.

	$[\alpha]^{25}$ nm					
Crystallization	589	578	546	436	365	conc^{n} in acetone
1.	+160.0	+164.0	+194.0	+350.0	+598.0	1.00
2.	+161.3	+168.9	+195.3	+352.8	+610.4	1.006
3.	+168.3	+177.0	+206.0	+368.5	+635.4	1.03
4.	+170.4	+180.0	+210.7	+390.2	+651.6	1.02

(Wren and Williams, 1916, d-Phenylsuccinic acid $[\alpha]_D$ + 168 in acetone)

The mother liquors from the formation of (+)-phenylsuccinic acid were evaporated under reduced pressure to yield white/yellow crystals. Three recrystallizations from water yielded (-)brucine salt of l-phenylsuccinic acid.

	$[\alpha]^{25}$ nm					
Crystallization	589	578	546	436	365	conc^{n} in acetone
1.	-58.0	-59.0	-71.0	-145.0	-300.0	1.00
2.	-71.0	-73.0	-81.0	-163.0	-331.0	1.00
3.	-73.5	-76.5	-90.2	-187.3	-377.5	1.02
4.	-74.0	-76.3	-87.1	-176.1	-365.9	1.02

Acidification of the salt (15.3 g) with c. HCl followed by ethereal extraction yielded a cream crystalline product. Three further recrystallizations from water yielded (-)phenylsuccinic acid (2.0 g) to constant rotation.

Crystallization	$[\alpha]^{25}$ nm 589	578	546	436	365	conc^{n} in acetone
1.	-138.0	-143.8	-166.0	-304.1	-504.8	1.04
2.	-156.0	-154.0	-171.0	-306.0	-523.0	1.00
3.	-169.6	-177.4	-205.7	-367.0	-628.3	1.06
4.	-168.0	-176.4	-206.6	-367.9	-631.1	1.02

(Wren and Williams, 1916, l-phenylsuccinic acid $[\alpha]_D$ -173.3)

3. Introduction

Since traditional methods of resolving racemic mixtures, ie fractional crystallisation of diastereomeric salts (as already described) are relatively difficult, time consuming and limited in applicability, there has been tremendous impetus in recent years to develop efficient liquid chromatographic techniques for such separations.

In order to achieve separation of enantiomers it is necessary to use some kind of chiral discriminator eg, a chiral stationary phase or a chiral additive to the mobile phase.

A third possibility is an indirect separation, where the enantiomers are converted to a mixture of diastereomers via a suitable chemical reaction using a chiral reagent. The diastereomers can then be separated by reverse or normal phase chromatography. There are, however, two major drawbacks to this technique.

1. It is not always possible to maintain or even obtain the necessary reagents with very high optical purity.

2. Enantiomers have quite different rates of reaction when reacted with another chiral molecule (Mislow and Raban, 1967) resulting in the production of two diastereomers of differing proportion to the starting enantiomeric pair.

These factors do not, however, eliminate the need for derivatisation which may still be necessary if the compounds of interest have poor chromatographic properties eg, amines and carboxylic acids, whereas amides generally chromatograph well.

3.1 Chiral Mobile Phase Additives (Dobashi and Hara, 1983; Tscherne and Capitano, 1977)

Using this technique racemic mixtures are resolved because of differences in the stabilities of the diastereomeric complexes so formed in the mobile phase. eg, solubility or binding to the achiral stationary phase.

Although not chiral, transition metal ions including Al(II), (Gil-Av et al, 1980) Ni(II), Zn(II) and Cd(II) (Lepage et al, 1979) have been used. If the formation of a diastereomeric tertiary complex between the metal ion and the enantiomers results in one of the ligands forming a stronger mixed complex then resolution will be achieved. Their applicability is, however, limited to the separation of racemic mixtures of amino acids eg l-proline (Gil-Av et al, 1980).

Diastereomeric ion pairs formed between a charged solute and a chiral counter ion of opposite charge may show different chromatographic properties. The counter ions that have been employed include d-10 camphorsulphonic acid (Petterson and Schill, 1981) quinine, quinidine and other cinchona alkaloids (Petterson and No, 1983). Mobile phases of low polarity eg, dichloromethane are preferential and the presence of water even in very low concentrations has been shown to have an adverse effect on separation.

Liquid-liquid chromatography can be utilised using a sparingly soluble optically pure reagent in the mobile phase eg, (+)-dibutyltartrate for the separation of chiral amines e.g. ephedrine (Petterson and Stuurman, 1984).

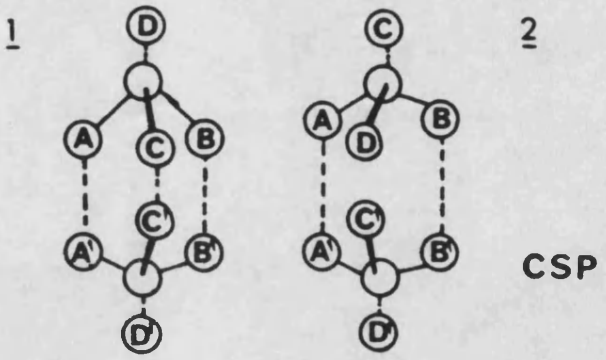
3.2 Chiral Stationary Phases (CSP) (Reviews: Souter, 1985; Dappen et al, 1986; Lochmuller and Souter, 1975)

Many chiral stationary phases are now commercially available. Two types have been used in this work, namely cyclodextrin columns and the α_1-amino glycoprotein (Enantiopac) column.

3.2.1. Pirkle Type (Wainer and Doyle, review, 1984)

Pirkle columns were developed on the basis of the three point chiral recognition model (Dalgleish, 1952) which proposed that chiral recognition required a minimum of three simultaneous interactions between a chiral stationary phase and a solute. These could be π - π bonding, H-bonding or Van der Waals interactions - but at least one must be stereochemically controlled in either an attractive or repulsive sense.

e.g.



In this example, enantiomer 1 interacts with the chiral stationary phase (CSP) at three sites i.e. A--A', B--B', C--C'. Its mirror image (enantiomer 2), however, only interacts at two sites. If the C--C' interaction is attractive then enantiomer 1 will be retained longer than 2, if repulsive then vice versa and if the C--C' interaction is only minimal or non-existent then no separation will be seen.

One of the CSP's developed by Pirkle uses R-N-(3,5-dinitrobenzoyl)phenylglycine bound to a γ-aminopropyl packing either ionically or covalently.

i.e.

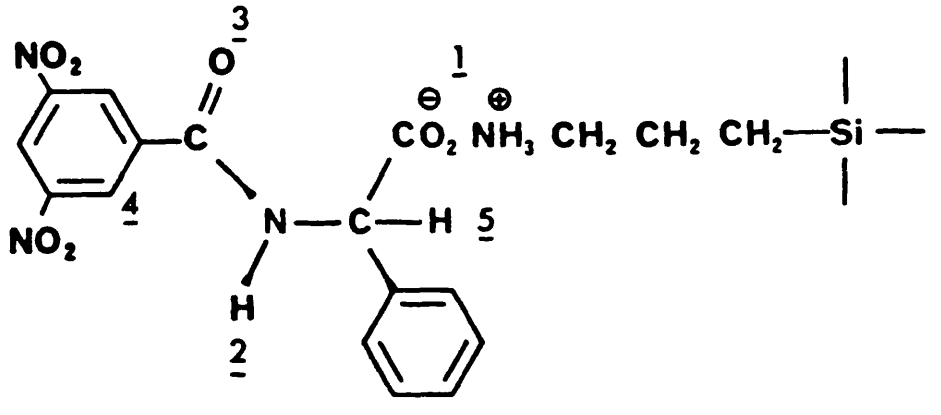


Fig 3.2 Ionically bound (R)-N-(3,5,dinitrobenzoyl)phenylglycine

This CSP has a number of possible sites for interaction:-

Site 1. The dipole formed by the amide linkage between the 3,5-DNB moiety and the phenylglycine.

Site 2. The amide hydrogen - available for hydrogen bonding.

Site 3. The amide carbonyl - available for hydrogen bonding.

Site 4. The 3,5-DNB ring (electron deficient) - available for $\pi - \pi$ bonding with other aromatic rings.

Site 5. The carbonyl and phenyl groups on the phenylglycine moiety which can interact with solute either attractively or repulsively (because of its steric bulk).

The many combinations of these sites provide a large number of ways for the CSP to interact with the solute, as well as the possibility of interaction with a wide range of molecules.

There are now many different varieties of Pirkle-type stationary phases ; both π - electron acceptor and donor groups are used. The large number of possible interactions give a Pirkle-type CSP its broad applicability. Examples of compounds resolved using this type of CSP include numerous enantiomeric amides (derived from amines or acids), alkyl carbinols, sulphoxides, lactones (Pirkle et al, 1980; Pirkle and House, 1979), propranolol analogues (Pirkle et al, 1981) and amino alcohols (as oxazolidines) (Wainer et al, 1986). Preparative resolution of a wide range of racemates has also been reported (Pirkle and Finn, 1982).

3.2.2. Chiral Cyclodextrin Bonded Phases

This CSP consists of cyclodextrin host molecules covalently bound to 5 micron silica gel via a ten atom spacer (Astec Informer, 1987). The coupling contains no nitrogen or Si-O-C linkages and is therefore hydrolytically stable.

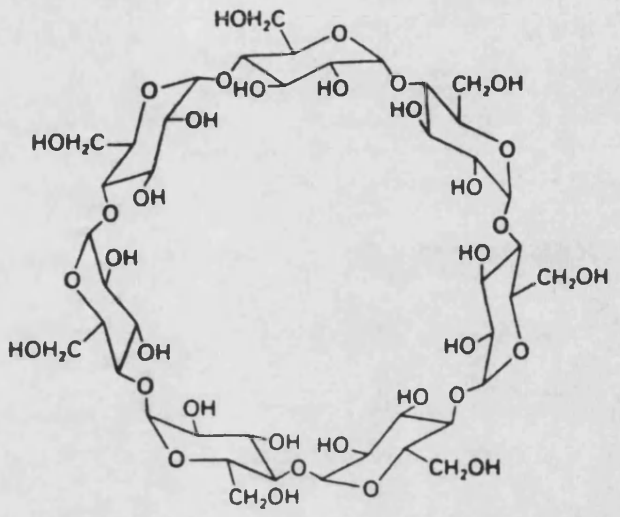


Fig 3.3 Chemical structure of β cyclodextrin molecule.

Cyclodextrins (CyD) are cyclic oligosaccharides composed of six (α), seven (β), or eight (γ) glucopyranose units, linked by α-1-4 bonds (Szejtli, 1982). Their structure is unique in that it resembles a truncated cone, with both ends open. The larger opening of the cone is rimmed with 14 secondary hydroxyl groups (in the β CyD) with C-2 in a clockwise direction and C-3 anti-clockwise. Thus the hydroxyl groups of adjacent glucopyranose units form hydrogen bonds which stabilize the shape of the molecule (Szejtli, 1982). The smaller opening is rimmed with the more polar primary hydroxyl groups (7 in total).

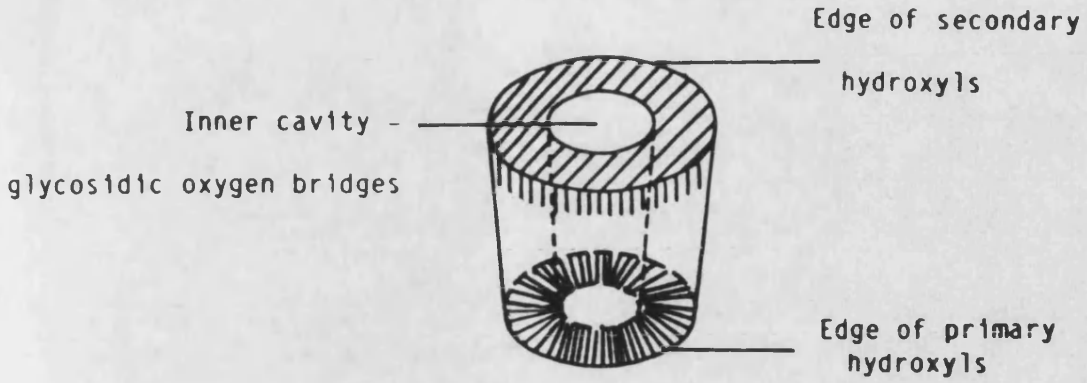


Fig 3.4 The molecular shape of CyD showing the central cavity.

The interior of the cavity contains no hydroxyl groups and is relatively hydrophobic overall. The result is a molecule with a hydrophobic centre and relatively hydrophilic outer surface. Consequently such molecules are able to complex a variety of water insoluble or sparingly soluble molecules.

The ß-cyclodextrins have been more widely applied (Armstrong, 1984) than α or γ because of their optimum size for inclusion-complex formation e.g. biphenyl and somewhat larger compounds may be accommodated in the ß but not in the γ polymer.

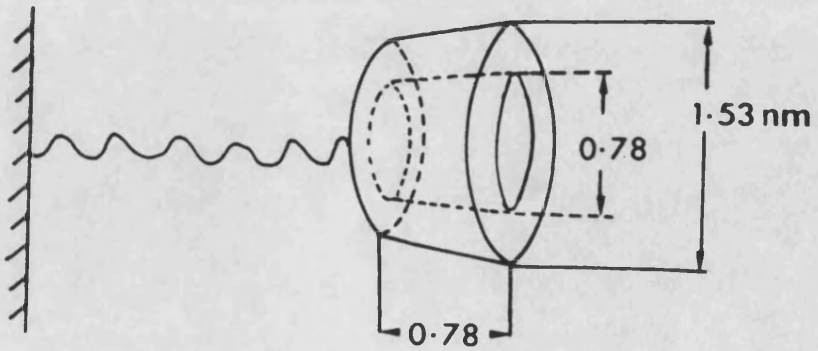


Fig 3.5 Representation of the size and shape of the ß CyD as it exists attached to a solid support.

Each glucose unit contributes five chiral centres, thus β-CyD contains 35 chiral centres. Guest solutes can interact through Van der Waals forces and also via Hydrogen bonds, with the hydroxyls at the mouth of the cavity if the chiral solute has the suitable polar sustituents.

In aqueous solution the slightly apolar CyD cavity is occupied by the water molecules which are energetically unfavoured (polar-apolar interaction) and are therefore readily substituted by the appropriate guest molecules which are less polar than water. Inclusion selectivity is formed only in the presence of water or a combination of water and organic modifiers such as DMSO, DMF, acetonitrile or alcohols. These organic modifiers tend to compete with all solutes for the prefered location in the hydrophobic cavity. Therefore increasing the concentration of an organic modifier will decrease retention. Generally, the binding strength of ionic species to the CyD is less than for the corresponding neutral species. Thus the efficiency of separations has been substantially increased in some cases by the use of buffers (Beesley, 1985), within the allowable pH range of 4.0 to 7.5.

Successful resolutions have been achieved using bonded CyD columns for a variety of compounds including geometric isomers of tamoxifen (Armstrong et al, 1987), structural isomers (Armstrong et al, 1985; Armstrong and Demond, 1984), and many drug enantiomers (Armstrong et al, 1986; Hinze et al, 1985).

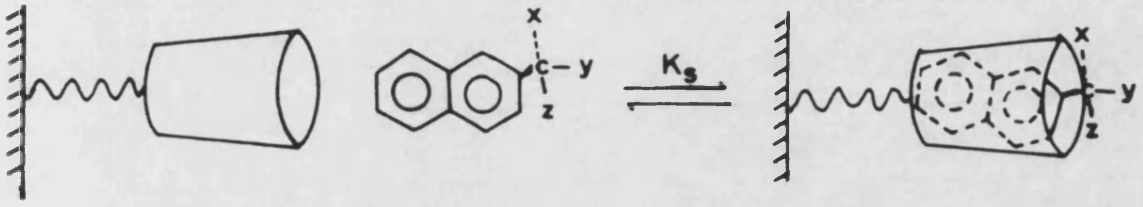


Fig 3.6 Schematic diagram of CyD bound to a silica gel support and reversibly forming an inclusion complex with a chiral molecule.

3.2.3. Protein Type

Interaction between acidic and basic drug compounds and proteins is well known. The proteins involved are albumin, which is the primary binding protein for acidic drugs and α_1-acid glycoprotein (AGP), which primarily binds basic drugs. These proteins are polymers composed of naturally occurring chiral amino acids and the binding that occurs can often be stereospecific.

This effect was first used for chromatographic separations by Stewart and Doherty (1973) who succeeded in resolving D and L tryptophan on bovine serum albumin (BSA) bound to agarose. Allenmark (1983) and Hermansson (1983) have developed bonded phase columns that take advantage of the albumin and AGP interactions respectively.

The separation mechanism of protein columns is not clearly understood. The separation appears to take place through differential interactions of enantiomers via a combination of hydrogen bonding, Van der Waals mechanisms, ion-pairing (Schill et al, 1986[1]) and steric effects (Allenmark et al, 1984).

Protein columns are demanding in that the chiral separation depends on the chromatographic conditions i.e. pH, ionic strength, organic modifier concentration (charged or uncharged) and temperature (Schill et al, 1986[2]). Used in the traditional reversed phase mode with aqueous buffered mobile phases, the recommended values are pH 3.0-7.5, ionic strength 0-500mM, organic modifiers up to 5% of 1- or 2-propanol or 0-10mM tetrabutylammonium bromide, temperature up to 35°C and a maximum flow-rate of 0.3ml/min.

The primary advantage of protein columns is their wide aplicability to enantiomeric separations of drug compounds (Wainer et al, 1986; Hermansson, 1985; Hermansson and Eriksson, 1986). Analysis of biological fluids is made simpler by the fact that aqueous mobile phases are used. Due to the very low flow rate, protein columns require more time than other types of chiral phases, resulting in broader peaks and poor sensitivity. This may limit their use for low dose drugs in biological fluids.

3.3 The Choice of Detector

Ultra-violet spectroscopy is the typical choice of detector for HPLC. UV detection can be used in the separation of enantiomers using HPLC - providing the compound has a suitable chromophore.

The main disadvantage of UV detection is that it discloses nothing about the nature of the separation between the two chiral components. It is not a specific detector for chiral separations and often the presence of impurities can hinder the analysis. During the course of the development of the separation it is not possible to detect whether the leading edge or the tail of the peak is enriched with one enantiomer. A sample which appears as one peak may well be only partially resolved.

In the separation of enantiomers it is not possible to determine the order of elution using UV detection. This must be derived from semi-preparative techniques and optical rotations using a polarimeter.

UV detection does, however, have its advantages. Once the enantiomers have been resolved and the peaks assigned, then providing there is sufficient resolution, quantitation, giving weight for weight concentrations, can be obtained using values of either peak height or peak area.

There are two other types of detectors available, which provide more peak information in the separation of optical isomers:-

1. Polarimeters, fitted with small volume flow cells are available. These detect the change in optical rotation as the peaks are eluted. They are however, very limited, since the degree of rotation varies enormously from one compound to the next. A compound with a very low specific rotation would have a very poor limit of detection using a polarimeter and quantitation of for e.g. 0.5% w/w of one of enantiomer in the other , may well be impossible. Certainly for development work the polarimeter would provide more information about the separation, than a UV detector, but its application is limited.

2. A more sensitive and versatile detector is one that utilises the phenomenon of Circular Dichroism (CD). A beam of plane polarised light may be considered to be made up of a left and right circularly polarised component. Optical rotation will be observed when a medium transmits the two circularly polarised components with unequal velocity. (see also 2.6.)

If in addition to unequal velocity, there occurs unequal absorption of left and right circularly polarised light, the emergent light will be elliptically polarised and this unequal absorption is referred to as Circular Dichroism (CD).

A CD detector measures the optical properties of eluting components and because the phenomenon is derived from light absorption, sensitivity is in the same order of magnitude as conventional UV/HPLC detection. It is therefore a far more versatile and sensitive technique than polarimetry.

The detector simultaneously measures the UV absorption and CD of the eluent passing through the flow cell. At a particular wavelength, the two enantiomers of a racemic mixture produce a CD peak of equal and opposite signs.

Providing the chromatographic peak consists of two partially resolved enantiomers, the CD trace is a composite of two overlapping peaks of opposite sign. Therefore, although by absorption there appears to be no resolution - the peak is resolved when the CD is measured (Salvadori et al, 1984).

3.4. Optimization of the Separation

Throughout the HPLC development work the following parameters are employed to describe the quality of separation:

Capacity factor $K' = \frac{(T_R - T_0)}{T_0}$

T_0-void time

T_R-retention time of the test compound

Separation factor $\alpha = \frac{K'_1}{K'_f} > 1$

K'_f capacity factor for the first eluted enantiomer.

K'_1 capacity factor for the last eluted enantiomer.

Resolution $Rs = \frac{T_1 - T_f}{0.5\ (W_1 - W_f)}$

T_1 and T_f represent elution times (in minutes)

W_1 and W_f represent peak widths at baseline

For a racemic mixture a value of $R > 1.5$ is equivalent to baseline separation.

3.5. Results and Discussion

3.5.1. Mobile phase additives

In principle, the technique of using di-p-toluoyl-tartaric acid to discriminate between R and S chlorpheniramine should also be transferable to chromatographic systems. Diastereomeric ion pair formation should enable separation to be achieved.

Attempts to achieve this practically proved unsuccessful. A mobile phase of AcN (25%) and water containing 5mM or 1mM of di-p-toluoyl-tartaric acid meant that , using a UV detector, the signal was off-scale. Reducing the concentration of di-p-toluoyl-tartrate to 0.1mM enabled the signal to be brought on scale but the peaks were too broad to be of any value. An alternative to this was to use similar mobile phase conditions but use electrochemical detection. This method looked promising and is still under investigation (Jefferies, unpublished).

Petterson and Schill (1981) reported the separation of enantiomeric amines using ion-pair chromatography, involving the use of a weaker chromophore d-10 camphor sulphonic acid (d-10 CSA) as the counter ion. Although use of this acid in the resolution of the antihistamines had not proved successful-it was still considered worthy of evaluation by HPLC techniques.

It was proposed (Petterson and Schill, 1981) that stereoselective association of the racemic amines (β-blockers) and d-10 CSA occurred via diastereomeric ion pair formation. These ion pairs were believed to have structural differences, substantial enough to distribute themselves differently between the organic mobile phase and the stationary adsorbent.

Again the three point interaction (Dalgleish, 1952) necessary for stereoselectivity, was thought to be important.

The system used involves a non polar stationary phase (Hypersil 5 ODS) and a mobile phase of greater polarity (DCM-AcN) containing d-10 CSA. Initial trials at Bath with a mobile phase of DCM-AcN 1% containing 5 or 10 mM d-10 CSA showed no resolution and rather broad peaks - due possibly to free base binding to uncapped silanol groups on the column.

Addition of a competing base (Lim et al, 1985) sharpened the peaks and enabled some slight separation to be seen. Optimum enantiomeric resolution was obtained with a ratio of 2:1 d-10 CSA to the competing base, diethylamine (DEA) (Table 3.1).

Compound (as maleates)	K_1	K_2	α	[DEA]
chlorpheniramine	0.67	-	-	
carbinoxamine	0.67	-	-	1.25mM
dimethindene	0.67	-	-	
chlorpheniramine	0.52	0.67	1.29	
carbinoxamine	0.62	0.81	1.31	2mM
dimethindene	0.51	0.66	1.29	
chlorpheniramine	0.44	0.57	1.30	
carbinoxamine	0.39	0.52	1.33	2.5mM
dimethindene	0 44	0.57	1.30	

Mobile phase: DCM:AcN 1%, containing 5mM d-10 CSA.

K- capacity factor

α- separation factor

K value for maleic acid =1.17

Table 3.1. shows the effect of increasing amounts of DEA on the K and α values of the test antihistamines.

Table 3.1 shows that with a low concentration of DEA there is no resolution. On increasing the amount of DEA, the retention time and thus the capacity factor is reduced. The results for the separation factor, α, are deceptively good. Since the capacity factor (K) values are low only a small difference between the two will result in an acceptable α value. Visually the separation was poor.

Trials using triethylamine (TEA) as the competing base were not successful. TEA appeared too strong and the retention times were shortened again.

Initial studies to find a mobile phase of choice included water as one of the sustituents. All the attempts using water failed and no separation was seen. Similar effects for other H-bonding agents have also been reported (Petterson and Schill, 1981). They proposed that this negative influence is probably due to the interaction with hydrogen bonding groups of the ion pair components, which then decreases the bonding strength within the ion pair.

The retention mechanism involved would appear to be more than simple partitioning of antihistamine-d-10-camphorsulphonate ion pairs between the mobile and stationary phases. In the absence of competing base the retention times were long and erratic, but in the presence of increasing amounts of competing base the K and α values decreased. This suggests a mechanism similar to that of Lim et al (1985) i.e. DEA-d-10 CSA ion pairs saturate the non-polar stationary phase through hydrophobic interactions (during equilibration). This gives rise to an equilibrium between free and bound ion pairs. Enantiomers of the antihistamines then ion exchange with protonated competing base.

3.5.2. Protein-Type Columns

Hermansson (1983, 1984) developed a chiral HPLC column based on the human plasma protein (α_1-acid glycoprotein, α_1-AGP). The stationary phase is based on N,N-diethylamino ethyl derivatized silica particles (10 μm) to which α_1-AGP has been adsorbed and immobilized. Within the pH range 3-7.5, the method of immobilization ensures that the α_1-AGP does not leak. Attempts were made in this laboratory to prepare an α_1-AGP column as directed (Hermansson, 1983; Herman et al, 1981) but unfortunately the resulting column showed very poor resolution. Finally an Enantiopac (100 x 4 mm) cartridge column was purchased from LKB.

Following the details given by Hermansson, (1984) for chlorpheniramine, separation of a series of racemic antihistaminic bases including the pheniramines, carbinoxamine, dimethindene was undertaken (Method 1). The results showed satisfactory separation of only a few of the compounds under test. The retention times however, were very long and about 2 hours were required for some separations to be completed.

The results (Table 3.2) showed best separation for RS pheniramine, giving a resolution factor Rs of 1.50 i.e. baseline separation. The inclusion of a halogen atom into the pheniramine structure has a detrimental effect on the resolution. RS chlorpheniramine maleate was resolved slightly (Rs 0.91) but RS brompheniramine showed no separation at all. Under these conditions the presence and increase in size of the halogen substituent was evidently reducing the stereo selectivity of the α_1-AGP towards the compounds.

In order to check the optical purity of chlorpheniramine more precisely a dehalogenation experiment (Shafi'ee and Hite, 1969) was performed using Pd/C as the catalyst and MeOH as the solvent, dehalogenation was complete after reaction overnight at room temperature (60psi). The RS pheniramine produced by this reaction on RS chlorpheniramine showed superior separation to chlorpheniramine and comparable separation to the original RS pheniramine. Similarly dehalogenation of resolved (-)-chlorpheniramine to (-)-pheniramine identified the first peak as the (-)-isomer and showed (-)-chlorpheniramine (from which it was derived) to be of high optical purity.

The only other compound to show any separation under these conditions was RS carbinoxamine (Rs 1.27) Table 3.2. It is interesting to note at this point that whereas in the pheniramines the dextro base has the greatest retention, in carbinoxamine the laevo base is retained longer. These are both the more active forms of the two compounds and share the same (S) configuration.

	T_1	K_1	T_2	K_2	α	Rs
RS pheniramine	51	7.5	118.5	18.7	2.50	1.50
RS pheniramine	37.5	5.25	84	13.0	2.48	1.15
(from chlorpheniramine)	40.5	5.75	97.5	15.25	2.65	1.73
(-)-pheniramine (from chlorpheniramine)	42.75	6.13	-	-	-	-
RS chlorpheniramine	80	15.0	105	20	1.33	0.91
	66	10	85	13.2	1.29	0.95
(+)-chlorpheniramine	-	-	105	20	-	-
	-	-	81	12.5	-	-
(-)-chlorpheniramine	80	15	-	-	-	-
	66	10	-	-	-	-
RS brompheniramine	100	15.7	-	-	-	-
RS carbinoxamine	84	13	126	20	1.54	1.27
	51	7.5	69	10.5	1.40	0.92
(-)[a]-carbinoxamine[b]	-	-	126	20	-	-
(+)[a]-carbinoxamine[b]	84	13	-	-	-	-

Mobile phase : 8MM Phosphate buffer containing 0.1M NaCl, 0.4% 4-propanol pH 6.9.

Footnotes

a run as tartrate salts

b sign of rotation refers to the base itself and not to the salt.

Table 3.2 Resolution details using Method 1.

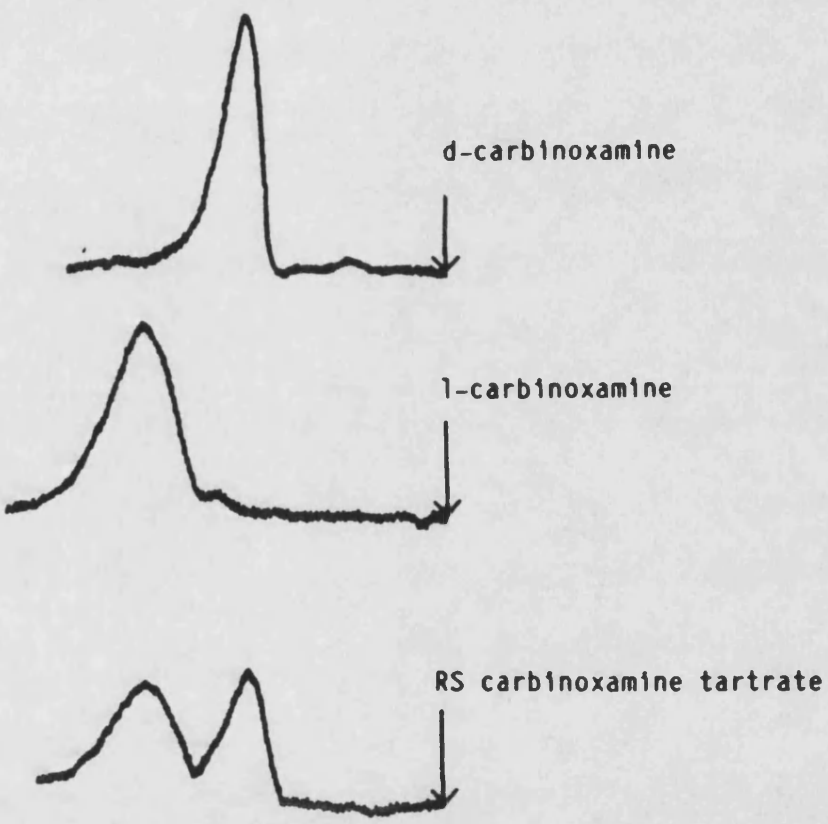


Fig.3.7. Chromatograms for RS carbinoxamine and its isomers using Method 1.

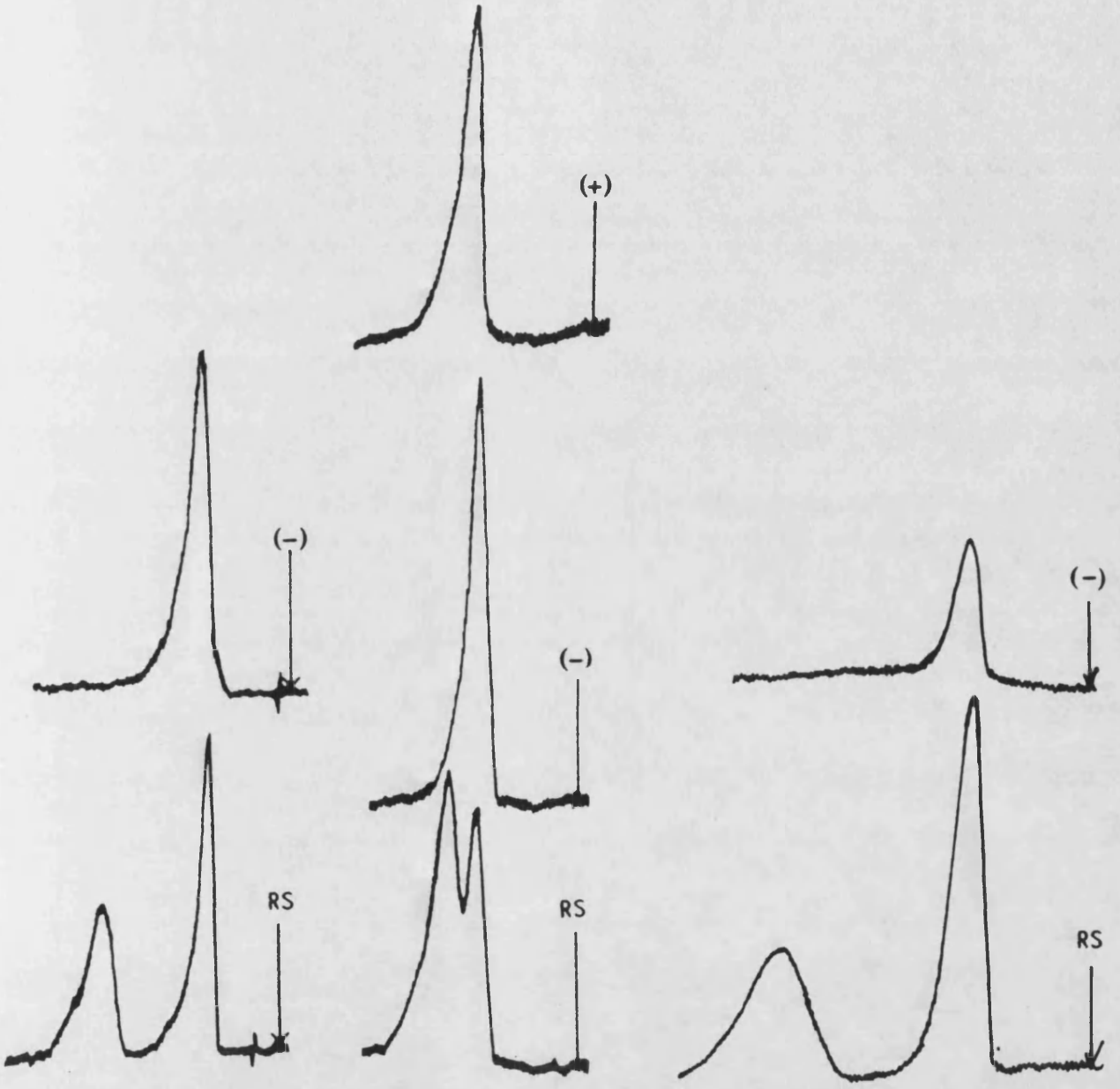


Fig 3.8. Chromatograms for the pheniramines, racemates and their isomers, using Method 1.

Since the retention times were so great, no more work was carried out under these conditions. Schill et al (1986[2]), using the Enantiopac column, but under differing conditions i.e. tetrabutylammonium phosphate instead of propanol/NaCl in the phosphate buffer, reported the resolution of chlorpheniramine (α 2.26) with greater success than that achieved previously.

These conditions were applied in the present work (Method 2). The resolution of the pheniramines increased greatly, RS pheniramine (Rs 2.76), chlorpheniramine (Rs 1.33) and even brompheniramine showed the beginnings of a separation (Rs 0.8). Again the (+)-isomer (S-configuration) was retained the longest. Resolution of carbinoxamine did not alter greatly (Table 3.3).

Attempts to resolve the other antihistamines of interest showed no advantage over Method 1. In the case of mebrophenhydramine, meclozine and hydroxyzine no resolution was seen. The use of a UV detector was possibly the reason - the λ max for these compounds is around 220-230nm and may be causing interference with solvent end absorption. Perhaps use of a CD detector would have been more applicable in this case.

Compound (as maleates)	T_1	K_1	T_2	K_2	α	Rs
RS pheniramine	18.3	4.1	60.0	15.7	3.84	2.76
RS pheniramine (from chlorpheniramine)	18.3	4.1	51.6	13.3	3.24	2.64
(-)-pheniramine (from chlorpheniramine)	20.45	4.67	-	-	-	-
RS chlorpheniramine	28.8	7.0	48.0	12.33	1.76	1.33
	29.4	7.17	47.4	12.17	1.70	1.10
(+)-chlorpheniramine	-	-	43.2	11.0	-	-
	-	-	45.0	11.5	-	-
(-)-chlorpheniramine	24.6	5.83	-	-	-	-
	27.0	6.50	-	-	-	-
RS brompheniramine	39	9.83	48.6	12.5	1.27	0.8
(+)-brompheniramine	-	-	47.1	12.1	-	-
RS carbinoxamine	23.4	6.08	37.8	9.5	1.56	1.00
	24.6	5.83	36.0	9.0	1.55	1.05
(-)[a]-carbinoxamine	-	-	33.6	8.33	-	-
	-	-	30.6	7.5	-	-
(+)[a]-carbinoxamine	24.6	5.83	-	-	-	-
	22.8	5.33	-	-	-	-

Footnotes as for Table 3.2

Table 3.3 Resolution details using Method 2.

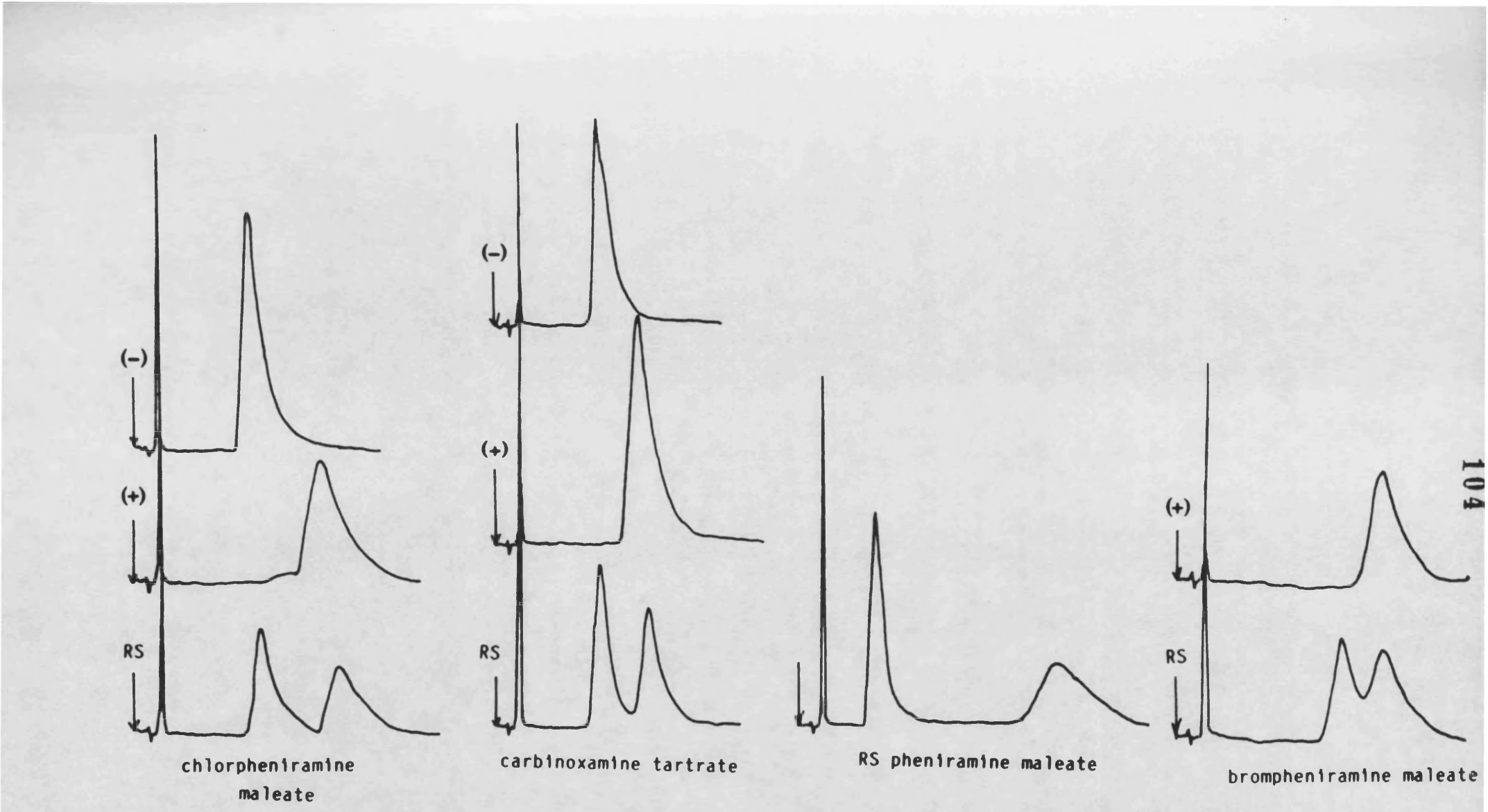
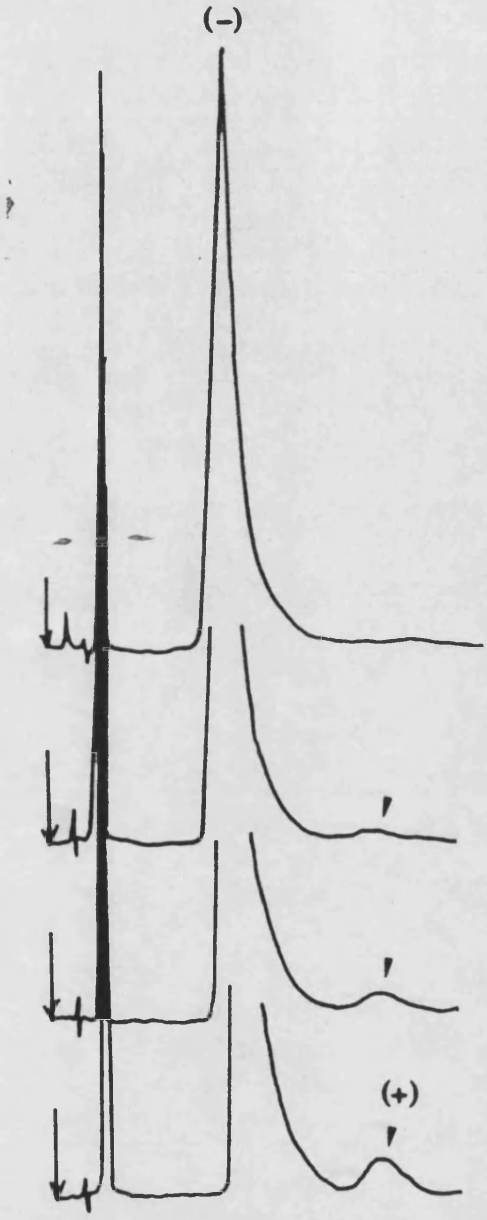


Fig 3.9 Chromatograms of the pheniramines and carbinoxamine (racemates and their isomers) using Method 2.

Using Method 2 a sensitivity experiment was carried out, whereby a solution of (-)-chlorpheniramine was spiked with increasing quantities of (+)-chlorpheniramine. The results showed that a 1% impurity of the (+)-isomer in the (-)-isomer was just visible on the chromatogram but that < 1% would not have been detected. Unfortunately no integrator was available at the time of completing this work.

The addition of tetrabutylammonium phosphate to the mobile phase reduced the retention greatly when compared to Method 1. (NaCl/propanol). The reason is probably that the charged modifier competes for both the chiral and non-chiral binding sites of the protein (Schill et al, 1986[1]). Since α_1-AGP is very acidic, at pH 7 (pH of the mobile phase) the protein molecule has a negative net charge. It is possible that the hydrophobic cationic additive competes with the solute for ionic binding to the negatively charged groups of the protein e.g. the sialic acid residues and also for binding at the hydrophobic part.

It should be noted that most of the separations have been carried out using a new Enantiopac column. Initial columns proved unsuccessful and the life of the columns was only ≃ 4 weeks. Improvements in the manufacture of these columns enabled better separation for the test compounds (LKB literature).



(-) chlorpheniramine maleate

1% (+) isomer in (-) isomer

5% (+) isomer in (-) isomer

10% (+) isomer in (-) isomer

Fig 3.10 Results of sensitivity experiment for chlorpheniramine isomers.

3.5.3 Cyclodextrin (CyD) Bonded Phases

Cyclodextrin (CyD) HPLC columns are commercially available, through Advanced Separations Technology, as Cyclobond I, II, and III, i.e. β, γ and α CyD respectively. The CyD moiety is bonded to a five micron spherical silica gel via a six to ten atom spacer. The 'original' Cyclobond columns consisted of CyD bound to silica particles whose particle size was not clearly defined. The first part of the work at Bath was carried out using this 'original' type of Cyclobond column. An improved β-CyD phase has since been introduced. These columns have nearly twice the efficiency and loading of the 'original' columns (Ward and Armstrong, 1986). Packed using better technology, these columns resulted in separations which were not able to be achieved on the 'original' column. The effect of these changes on the column will be discussed and comparisons, to the results obtained with the 'original' column drawn.

Trials to separate our test antihistamines (RS chlorpheniramine, RS carbinoxamine and RS dimethindene) using an 'original' Cyclobond I column and a mobile phase of methanol / water were initiated. At 90% water all the test samples eluted just after the solvent front (SF) and peak shape was unsatisfactory in the extreme. To suppress ionisation and enhance peak shape, the aqueous water phase was replaced with an aqueous ammonium acetate buffer (0.1M), pH6.

Effect of decreasing the organic phase content.

Using a mobile phase of 50% methanol: 50% ammonium acetate, pH 6, no separation of the test antihistamine peaks was seen. All the samples were run as their maleate salts dissolved in the mobile phase. The peak due to the maleic acid (identified by injection of pure maleic acid dissolved in the mobile phase) was retained for a shorter time than was carbinoxamine itself but for an equal length as dimethindene and chlorpheniramine. Decreasing the methanol content to 40% increased the retention time (R_T) and separated the maleic acid peak from that of chlorpheniramine and dimethindene but did not affect the resolution of the respective enantiomers. At 20% methanol, the separation of the enantiomers of carbinoxamine was beginning to be visible, R_T $\simeq$ 45 mins, α 1.02. The other samples did not show such promise.

The methanol content was maintained at this proportion and attempts to improve the separation continued by altering other factors.

Effect of temperature

Reducing the temperature from room temperature to 15, 10 and then 5 °C increased the retention time but again had little effect on the separation factor, α.

Effect of pH

Changing the pH from 6 down to 4 showed no appreciable change in peak shape, retention time or separation factor.

Effect of Ionic Strength

Increasing the ionic strength from 0.1-0.5M ammonium acetate should force the test compounds into the cavity resulting in an increase in the retention time. This effect was infact seen and the retention time increased to ≃ 100 mins. Although the peaks were still very broad, separation of the enantiomers was beginning to be seen, carbinoxamine and chlorpheniramine, α = 1.04.Further increase in ionic strength to 1M ammonium acetate, necessitated the inclusion of a silica guard column 'in-line' between the pump and rheodyne injector, to saturate the mobile phase before reaching the Cyclobond column. After equilibration overnight, the R_T for both carbinoxamine and chlorpheniramine had both increased further, R_T ≃ 140 mins accompanied also by an increase in the separation factor, α = 1.06.

Effect of Flow Rate

Reducing the flow rate from 0.5 to 0.3 ml/min under the above conditions had little advantage for carbinoxamine, the α value was now 1.09, but the peaks were just as broad.

Preliminary trials using a γ CyD column (Cyclobond II) were not encouraging but the β-acetylated column (Cyclobond I - acetylated) showed similar separation to the underivatised β.

Inclusion of an ion pair reagent, sodium perchlorate 0.1M, into the mobile phase, to prevent drug binding to the free silanol groups on the column, had the desired effect of reducing tailing and sharpening the peaks but the undesirable effect of worsening resolution.

Due to the lack of success with this 'original' column, an improved column was obtained. Using the same conditions as before (20% MeOH : 0.1M ammonium acetate, pH 4, room temperature), the R_T of carbinoxamine and chlorpheniramine had again increased to 150 and 170 mins respectively. The separation factors had similarly increased to α 1.05 and 1.09 respectively - but peak shape remained the same.

A new buffer system was now investigated to assess the effect on peak shape. The new mobile phase was methanol : 1% triethyl - ammonium acetate (TEAA), pH 4.1. When the methanol content was reduced from 40 to 20%, separation became visible and peak shape was enhanced to give the following results - carbinoxamine α = 1.05 and chlorpheniramine α = 1.09 (Fig 3.11). To increase separation and peak shape further, the methanol organic phase was replaced by a more polar organic phase i.e. acetonitrile.

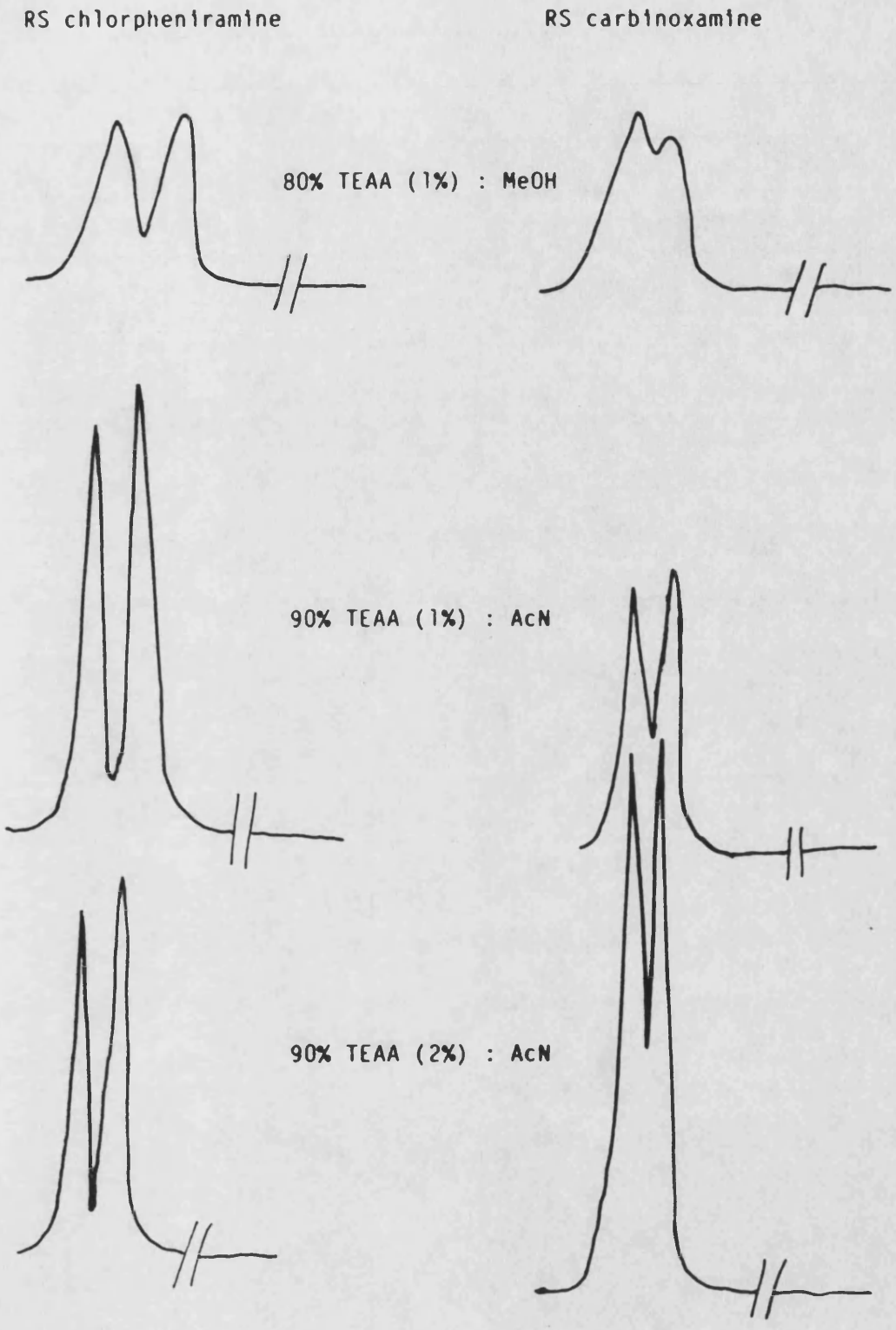


Fig 3.11 Trace chromatograms for the resolution of RS chlorpheniramine and RS carbinoxamine using differing mobile phases.

Armstrong et al (1986) published data for the resolution of chlorpheniramine (α 1.07, Rs 1.51) using a β-CyD column. These results were comparable to those obtained at Bath, as previously described using a 20% MeOH : 80% TEAA (1%) mobile phase. Using Armstrong (1986) conditions i.e. 85% TEAA (1%), pH 4.1 : acetonitrile, but at a slower flow rate (0.5 ml/min), the resolution of chlorpheniramine showed a comparable separation factor α 1.09, but the resolution was only Rs = 1.00 Attempts to increase the resolution continued.

Decreasing the organic phase to 10% increased the separation of chlorpheniramine (α = 1.11) and carbinoxamine (α = 1.08) still further, although baseline separation had still not been achieved. An increase in the strength of TEAA used, from 1-2% sharpened the peaks still further but still did not give baseline separation Rs > 1.5. This was, however, the mobile phase used for future work. (Table 3.4, Fig 3.11)

Compound (as maleates)	T_1	K_1	T_2	K_2	α	Rs
85% TEAA (1%) : AcN						
RS chlorpheniramine	22.5	2.75	24.3	3.04	1.11	1.00
RS carbinoxamine	24.3	3.04	25.5	3.25	1.07	0.83
90% TEAA (1%) : AcN						
RS chlorpheniramine	42.8	6.1	46.5	6.75	1.1	1.14
RS carbinoxamine	45.3	6.5	48.0	7.0	1.08	0.83
(-)-carbinoxamine	-	-	48.8	7.1	-	-
(+)-carbinoxamine	45.8	6.63	-	-	-	-

Table 3.4 Chromatographic features for carbinoxamine and chlorpheniramine using an improved Cyclobond I column.

The results (Fig 3.12, Table 3.5) show that only successful resolutions were achieved with the halogenated pheniramines and carbinoxamine. No separation was seen for dimethindene (20) although it was retained by the CyD column ($R_T \simeq 30$ mins). Its structure, although containing two aromatic substituents linked to the chiral centre, does not appear to be sufficient for stereoselectivity. It is unlikely that the overall shape of the molecule hinders its inclusion into the CyD cavity since ^1H NMR data provides clear evidence for the formation of an inclusion complex (4.4.1).

No resolution was seen for mebrophenhydramine (11), meclizine (13) or hydroxyzine (15). This may again have been due to the choice of detector and the problem of solvent cut-off. As with dimethindene ^1H NMR provides evidence for the formation of an inclusion complex between mebrophenhydramine and β CyD (4.8).

The pheniramine series (17) showed an interesting reversal of the trend seen with the α_1 AGP column in that the presence and increase in size of the halogen sustituent enhanced the stereoselectivity. This reinforced Beesley (1987) who stated that halogens have a very large affinity for the CyD cavity.

Interesting also is the fact that again it is the more active isomer of each of the two compounds that is retained the longest i.e. (+) pheniramines and (-) carbinoxamine - both of S configuration. This confirmed some preliminary studies using 'docking experiments' in molecular graphics which showed S-carbinoxamine to form more stable inclusion complexes.

Compound (as maleates)	T_1	K_1	T_2	K_2	α	Rs
RS chlorpheniramine	37.5	5.25	40.6	5.75	1.10	1.33
(+)-chlorpheniramine	*	*	40.5	5.75	-	-
(-)-chlorpheniramine	37.6	5.25	-	-	-	-
RS brompheniramine	26.9	7.83	29.9	8.92	1.14	1.52
(+)-brompheniramine	-	-	30.0	9.0	-	-
(-)-brompheniramine	25.7	7.5	-	-	-	-
RS pheniramine	19.2	2.2	19.52	2.25	1.03	-
RS carbinoxamine	41.49	5.92	43.69	6.25	1.06	1.00
(-)-carbinoxamine	-	-	43.8	6.29	-	-
(+)-carbinoxamine	41.7	5.95	-	-	-	-

Mobile Phase: 90% (TEAA)(2%) : AcN.

Footnotes

* 3% impurity peak found

Fig 3.5 Chromatographic results for test compounds after increasing the strength of TEAA.

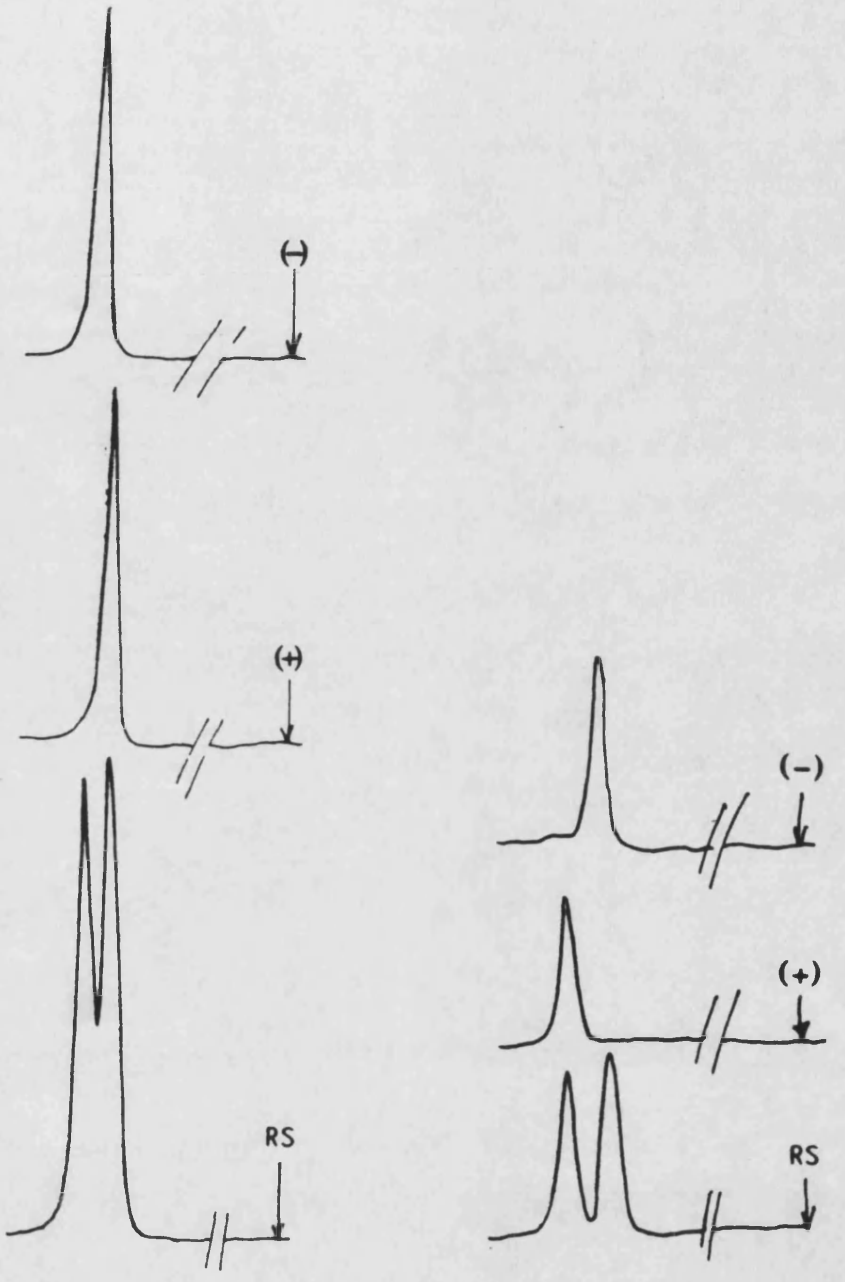


Fig 3.12 Selection of chromatograms showing the resolution of the enantiomers.

Sensitivity experiments carried out using (-) chlorpheniramine spiked with (+) chlorpheniramine, showed the level of detection to be at 0.5% of one isomer in the other (Fig 3.13).

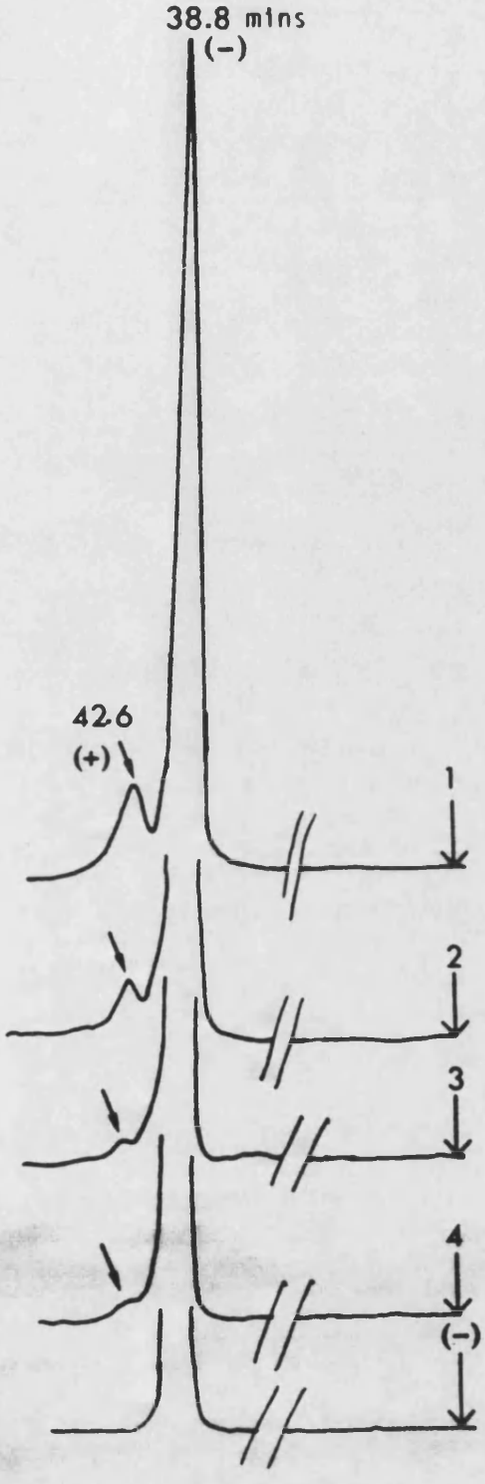
Such tests using carbinoxamine, (-) isomer spiked with the (+) isomer were not as successful and under these conditions only a 1% impurity of (+) in the (-) could be detected (Fig 3.14).

The use of the cyclodextrin column has highlighted some points made earlier (Chapter 2) i.e. that crystallisation to a constant rotation is not 'an absolute indication of optical purity'. Although the chlorpheniramine maleates had constant rotations, equal but opposite in sign,

i.e.(+) chlorpheniramine maleate $[\alpha]_D^{24}$ +23.2 (c 1.2 in H_2O)

(-) chlorpheniramine maleate $[\alpha]_D^{24}$ -23.9 (c 1.08 in H_2O)

chromatographic techniques using the CyD column have shown that resolved (+) chlorpheniramine maleate does actually contain a 3% impurity of the weaker (-) isomer (perhaps this explains the 0.7 difference in the two α values). This is not as much of a problem as if the (-) isomer (weaker) had been contaminated with (+) isomer - but it may affect the human study results. HPLC results have shown the (-) isomer to be 100% pure (Fig 3.15).



	K_1	Integ	K_2	Integ
1	5.5	88.1	6.08	11.9
2	5.5	96.04	6.08	3.96
3	5.5	98.91	6.08	1.09
4	5.5	99.7	6.08	-

1ml stock solution (2mg/ml) (-)chlorpheniramine maleate was spiked with varying volumes of (+) isomer (2mg/ml)

1 100µl(+) ⟶ 1ml(-) = 10%

2 50µl(+) ⟶ 1ml(-) = 5%

3 10µl(+) ⟶ 1ml(-) = 1%

4 5µl(+) ⟶ 1ml(-) = 0.5%

Mobile phase : 90% TEAA (2%) : AcN

Fig 3.13 Chromatogram illustrating sensitivity experiment for (-) chlorpheniramine maleate spiked with the (+) isomer.

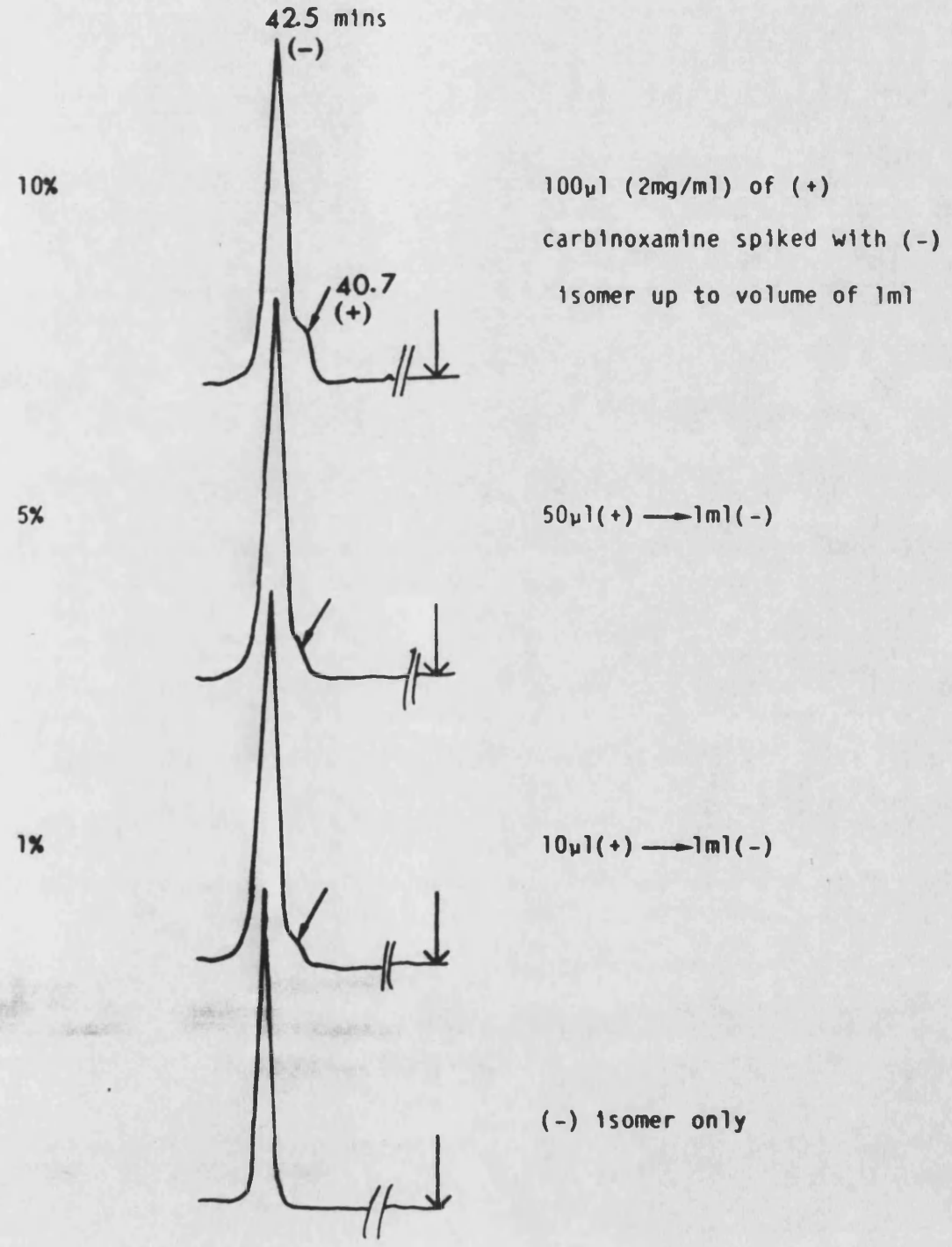


Fig 3.14 Chromatogram illustrating sensitivity experiment for (-) carbinoxamine maleate spiked with (+) isomer.

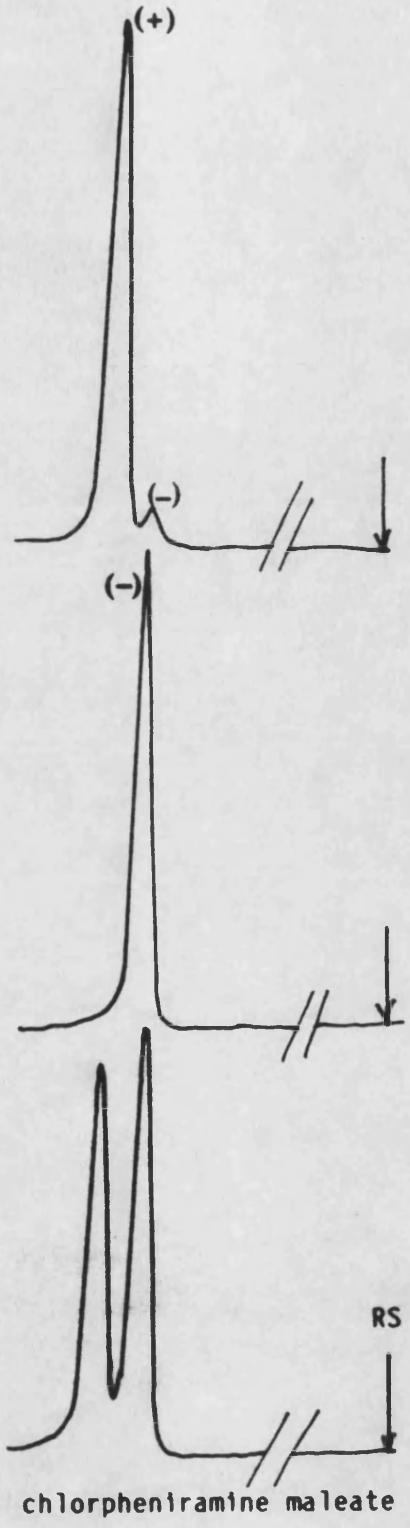


Fig 3.15 HPLC trace showing (-)chlorpheniramine to be 100% pure whilst the (+) isomer has a 3% impurity

3.6 Materials and Methods

3.6.1 Instrumentation

All measurements were carried out on an LDC / Milton Roy constametric 3000 pump linked to an LDC / Milton Roy spectrophotometer 3000 variable wavelength detector and a BBC SE12 chart recorder.

3.6.2 Materials

(RS)- antihistamines were supplied by the pharmaceutical industry as follows : dimethindene maleate (Zyma), carbinoxamine maleate (Wyeth Research UK), doxylamine succinate (Merrell Dow Pharmaceuticals), pheniramine maleate (A.H. Robins and Hoechst UK), chlorpheniramine maleate (Smith Kline and French Research), brompheniramine maleate (A.H. Robins), mebrophenhydramine HCL (Smith Kline and French Research).

3.6.3 Experimental Details

Enantiopac Column Work

Method 1 (Hermansson, 1984)

Column :	Enantiopac cartridge column
Mobile Phase:	8mM Na_2HPO_4 / $NaHPO_4$ buffer containing 0.1M NaCl, 0.4% 4-propanol, pH6.9.
Flow rate:	0.3ml/min.
Detection:	254 nm.
Injection volume:	10 µl.
Sample preparation:	≈2 µg salt dissolved in the mobile phase.
Temperature:	15°C.

Method 2 (Schill et al, 1986[2])

Column :	Enantiopac cartridge column
Mobile Phase:	0.2M phosphate buffer pH 7 containing 0.003M Tetrabutylammonium phosphate.
Flow rate:	0.3ml/min.
Detection:	254 nm.
Injection volume:	20 µl.
Sample preparation:	≈4mg salt dissolved in 25 ml mobile phase.
Temperature:	20°C.

Cyclobond I column

Column :	Cyclobond I 25cm column.
Mobile Phase:	90% TEAA (2%) : AcN.
Flow rate:	0.5ml/min.
Detection:	254 nm.
Injection volume:	20 µl.
Sample preparation:	0.02mg / ml in mobile phase.

Method for dehalogenation of chlorpheniramine

Reduction (50mg of 10% Pd-C, 22°C, 60psi, 50ml MeOH) of 5g chlorpheniramine (RS or levo) obtained from its hydrogen maleate was continued until absorption of H_2 had ceased (8-12 hours). The Pd-C was removed by fitration through celite. The filtrate was evaporated under reduced pressure to yield a purple oil of pheniramine. Formation of the hydrogen maleate (as Chapter 2) yielded a white crystalline solid (2.95g 46%). Evidence of the success of this method was by HPLC study as previously described and by ^{13}C-NMR which showed an increase of one CH aromatic resonance after dehalogenation.

UV absorption characteristics of compounds

It was necessary to check if the compounds possessed suitable UV absorption characteristics to enable a UV detector to be employed in the subsequent HPLC studies.

Solutions of the compounds (0.001%) in water were prepared. The UV absorbance or each solution was recorded on a Perkin Elmer 550S UV spectrophotometer from 200 - 300 nm wavelengths, using water as the blanks.

	λmax	A	$E^{1\%}_{1cm}$
Brompheniramine maleate	261	0.241	241
Carbinoxamine maleate	260	0.290	290
Chlorpheniramine maleate	261	0.279	279
Dimethindene maleate	257	0.641	641
Hydroxyzine DiHCl	230	0.733	733
Mebrophenhydramine HCl	225	0.704	704

The results show that most of the compounds (with the exception of mebrophenhydramine and hydroxyzine) have a λ max near 260nm and so a UV detector could be used in the HPLC analysis of the compounds. The wavelength of choice for the assay will, however, depend on the mobile phase as well as the compound being analysed.

UV detection may not be suitable for mebrophenhydramine or hydroxyzine since their λ max may be too low - detection at this wavelength may increase the interference from the solvent end absorption.

Chapter 4

Applications of cyclodextrins to chiral analysis by ^{1}H NMR

4.1 Introduction

The use of cyclodextrins (CyD) in forming inclusion complexes with medicinal agents is currently a topic of interest. The chief pharmaceutical interest relates to the possible stabilization (Anderson and Bundgaard, 1984[1], 1984[2]) and solubilization of included guest molecules and the improved drug release, absorbtion and bioavailability (Uekama et al, 1982) of poorly soluble drugs. Other applications (Szejtli, 1982) are in the reduction of side effects (Nambu et al, 1978), masking of unpleasant taste (Fujioka et al, 1983) and smells, the conversion of liquid drugs to a crystalline form and analytical separations.

Although there are several reports on the use of ^{1}H NMR spectroscopy in the study of such complexes (Nakajima et al, 1984), a recent paper (Greatbanks & Pickford, 1987) has drawn attention to the stereochemical value in the case of inclusion complexes formed with chiral guest molecules. Following their success with RS propranolol hydrochloride - trials at Bath between CyD (α, β & γ) and racemic antihistaminics were undertaken to illustrate the potential value of this data with regard to optical purity analysis and as a guide to whether the development of chromatographic separations of stereoisomers based on CyD (bonded as in Chapter 3 or in the eluate) was likely to be successful.

These results of ^1H NMR analyses, as will be shown, also provide information about the orientation of the guest molecule and its conformation within the host cavity. Evidence for the formation of an inclusion complex, between the CyD and guest molecule is provided by changes in the chemical shifts (and multiplicities) of signals due to both partners, while locations of such changes pinpoint the regions of the molecules implicated in the interaction.

4.2 ^1H NMR features of cyclodextrins in D_2O

The ^1H NMR spectrum of γ CyD was studied by Rao & Foster (1963) at 60 MHz who observed only the signal of a single kind of anomeric proton in the NMR spectrum. The ^1H NMR spectrum of α CyD was further studied by Wood et al (1977) at 100 and 220 MHz. This work confirmed that the C1 chair of glucose was not disturbed in the cyclic polymer.

C-1 Chair 1-C Chair

Fig 4.1 Shows the conformations of glucose (partial structures)

The work at Bath, using α-, β- and γ-CyD, was carried out at 270 or 400 MHz and thus provided better resolution of the signals than that of spectra previously reported. The ^{1}H NMR spectra of each CyD resembles that of their closest monomeric analogue, α-methylglucoside.

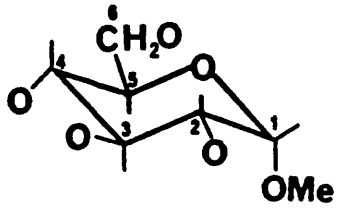


Fig 4.2 α-methylglucoside (partial structure)

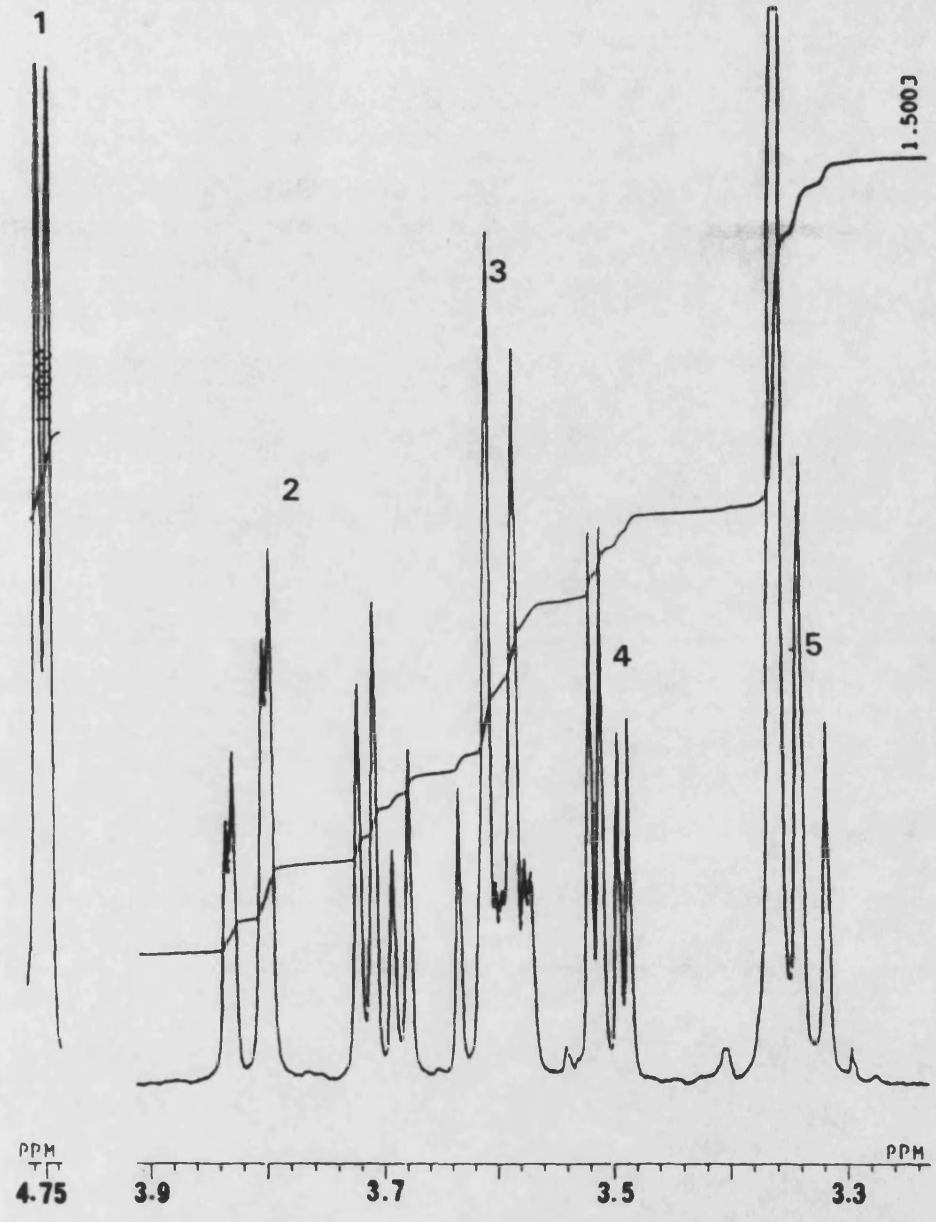


Fig 4.3 400 MHz ^{1}H NMR spectrum of α-methylglucoside run in D_2O

Only five of the seven proton signals were resolved. The lowest field proton, seen as a doublet (d) at 4.75 ppm, is due to the anomeric proton, H-1, because it is flanked by two deshielding oxygen atoms. The small coupling (≈4 Hz) is consistent with its equatorial conformation, showing an equatorial (eq)/axial (ax) coupling to the axial H-2 proton.

Peak number 4, at 3.51 ppm , is assigned as H-2 since this also showed a small eq/ax coupling, of 3.6 Hz, to the H-1 proton. In addition this doublet of doublets (dd) also shows a large ax/ax coupling, to H-3, of 9.5 Hz.

Peak number 5 (3.59 ppm) is assigned to H-4. This apparent triplet (t) signal (the lowest field line was obscured by the O-Me signal) is made up of two large ax/ax couplings (to H-3 and H-5) of 9 Hz.

Peak number 2 is assigned as the two non-equivalent H_2-6 signals. This eight line signal is typical of an AB system. Each half of the pattern includes a coupling (12.5 Hz) typical of a ^2J geminal coupling. In addition the two H-6 protons are coupled in differing degrees (2.6 Hz and 5.3 Hz) to H-5.

Peak number 3 is a compound signal due to the resonances of H-3 and H-5; H-3 gives a triplet at 3.61 ppm, due to two large ax/ax couplings of approximately the same magnitude (9 Hz), the highest field line overlapping with the H-5 resonance. The H-5 signal is more complex and all the peaks of the multiplet cannot be clearly analysed.

This analysis agrees with that of De Bruyn et al (1975), carried out at 300 MHz.

The analysis of the cyclodextrins by ^1H NMR (400 and 270 MHz) followed. At 400 MHz four (out of seven) proton signals of β-CyD were resolved.

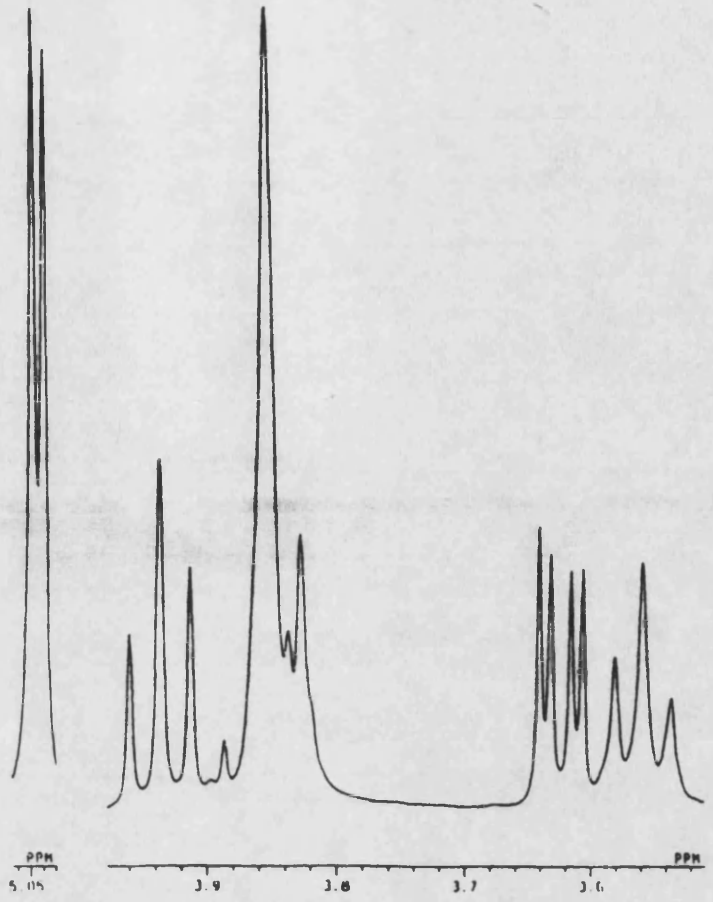


Fig 4.4 400 MHz spectrum of β-CyD run in D_2O

The analysis was aided by the assignments of α-methylglucoside and by the chemical shifts of the protons of α-CyD (Wood et al, 1977). The lowest field doublet (5.05 ppm) with the small coupling ≃4 Hz was assigned as the anomeric proton H-1. The H-2 proton was again visible as a doublet of doublets at 3.63 ppm showing a large ax/ax coupling to H-3 (9.8 Hz) and a smaller ax/eq coupling (3.5 Hz) to H-1. In the β-CyD spectrum H-4 and H-3 are both clearly visible as triplets at 3.56 and 3.94 ppm respectively. Protons H-5 and H_2-6 overlap to give a multiplet at ≃ 3.86 ppm.

Assignment of the two triplet signals was not immediately evident. The signal at 3.56 ppm assigned as H-4, was slightly broader than that at 3.94 ppm. One possible explanation of this is that the H-4 proton is showing long-range coupling to H_2-6.

A similar pattern emerged for the 400 MHz spectrum of α- and γ-CyD. See Table 4.1 for details.

It is interesting to note a downfield shift of all the protons of CyD compared to those of α-methylglucoside (Table 4.2)

Table 4.2 Downfield shifts (in ppm) of CyD protons in relation to those of α-methylglucoside

	glucose proton					
	1-H	2-H	3-H	4-H	5-H	6-H_2
β-CyD	0.30	0.11	0.33	0.22	(0.26)	0.03/0.15
α-CyD	0.28	0.10	0.35	0.25	(0.26)	0.03/0.15
γ-CyD	0.33	0.12	0.30	0.25	(0.26)	0.03/0.15

The downfield shift seen in the CyD moiety is most pronounced at H-1 and the two inner protons H-3 and H-5 (see Fig 4.5 for schematic diagram of CyD). As a result of this shift H-3 moves downfield of H_2-6 and is fully resolved and H-5 overlaps with the H_2-6 signal to produce a signal that is not fully resolved, as a complex multiplet.

Table 4.1: 400 MHz ^{1}H NMR characteristics in D_2O of three cyclodextrins and their monomer equivalent, α-methylglucoside[a]

Carbohydrate	1-H	2-H	3-H	4-H	5-H	6-CH_2
α-methylglucoside[b]	4.75	3.51	3.61	3.34	nr 3.59	3.82 dd(2.6,12)
(OMe s 3.36)	d(4)	dd(4,9.5)	t(9)	t(9)	ddd[c]	3.70 dd(5.3,12)
beta (β-)	5.05	3.62	3.94	3.56	e	e
cyclodextrin	d(4)	dd(3.5,9.8)		t(9.5)	bt(9)[b]	
alpha (α-)	5.03	3.61	3.96	3.59		
cyclodextrin[f]	d(3.5)	dd(3.4,10)	t(9.5)	t(9)	e	e
gamma(γ-)	5.08	3.63	3.91	3.59	e	e
cyclodextrin	d(3.7)	dd(3.4,9.5)		t(9.5)	t(9.5)	

Footnotes

a = chemical shifts in ppm from TMS using acetone (2.1 ppm) as external reference; multiplet separations (Hz) in parentheses; abbreviations s singlet, d doublet, t triplet, m multiplet, b broad.

b = cf 300 MHz study of Dr Bruyn et al (1975)

c = overlaps 3-H signal, only 2.6 Hz coupling resolved

d = broader than 3-H signal probably due to long range coupling to 6-CH_2

e = overlap to form a multiplet centred near 3.85 ppm

f = cf 220 MHz study of Woods et al (1977) and 400 MHz spectrum of Alston et al (1985)

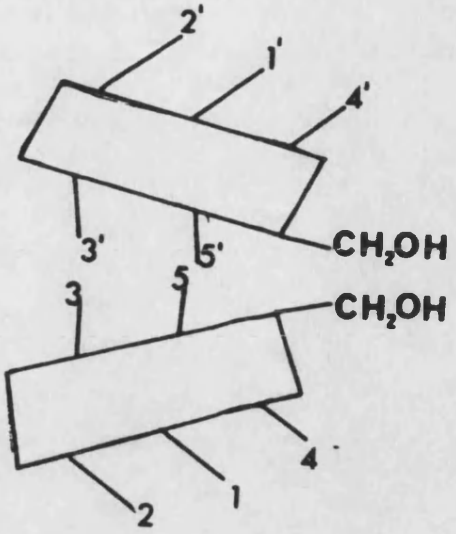


Fig 4.5 Cross section of the torus of a CyD, indicating the approximate position of the sugar protons

In the 270 MHz spectra, all the features of the 400 MHz spectra were apparent. However, the multiplet near 3.86 ppm, (i.e. the H-5 and H_2-6 signal) was less well resolved and the H-2/H-4 signals overlapped.

4.3 ^{1}H NMR studies of pyridyl compounds

Since several of the chiral antihistamines studied in this thesis are 2-pyridyl derivatives, analysis of the ^{1}H NMR spectra of pyridine and 2-ethylpyridine and their protonated maleate salts, in D_2O, were carried out to aid in the subsequent assignments.

4.3.1 Pyridine

The 270 MHz spectrum of the non-protonated pyridine molecule in D_2O and that of its protonated maleate salt show the same rank order of the signals but an increase in the resolution of the fine structure.

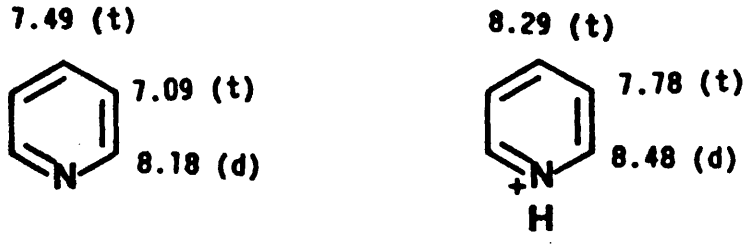


Fig 4.6 Chemical shift values (ppm) of non protonated and protonated pyridine run at 270 MHz in D_2O

The lowest field signal (maleate) seen as a doublet at 8.48 ppm is due to the protons, ortho to the ring nitrogen. The deshielding influence of this ring nitrogen is least at the proton meta to the nitrogen, this signal being seen as a triplet at 7.78 ppm. The triplet at 8.29 ppm, incorporating ortho and meta coupling was due to the proton para to the ring nitrogen.

4.3.2 2-Ethylpyridine

Although the commercial sample included impurity signals, the principal signals could be resolved and assigned on the basis of their chemical shifts and multiplicities. The lowest field peak (8.27 ppm) was a doublet (5Hz) and was assigned as the proton ortho to the nitrogen (H-6').

The H-4' proton signal was resolved at 7.64 ppm, showing ortho coupling (8 Hz) to H-3' and H-5' and meta coupling to H-6' (1.7 Hz). H-3' and H-5' signals overlapped near 7.18 ppm, but it was possible to identify the H-3' proton as a doublet (7.18 ppm, 8 Hz), and H-5' as a triplet (7.13 ppm, 8 Hz).

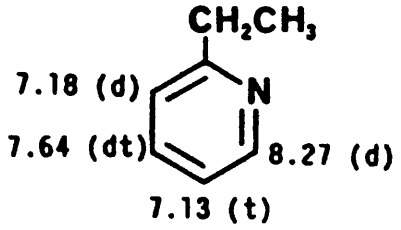


Fig 4.7 Chemical shift values (in ppm) of 2-ethylpyridine in D_2O, at 270 MHz

Thus in 2-substituted pyridines the rank order of the pyridyl protons is H-6' (lowest field), H-4', H-3' and H-5' (highest field).

The spectra of 2-ethylpyridine run in the presence of β-CyD showed much sharper peaks and clearly resolved the H-3' and H-5' proton signals (at 270 MHz).

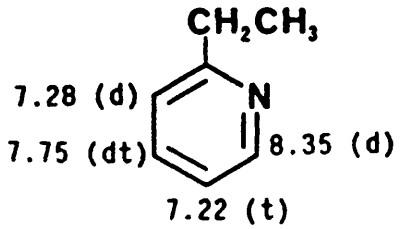
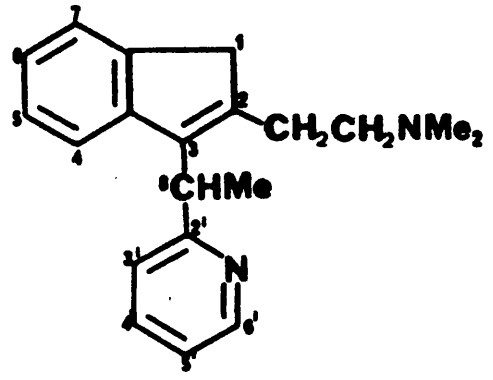


Fig 4.8 Chemical shift values (in ppm) of 2-ethylpyridine, in D_2O, in the presence of β-CyD (270 MHz)

^{1}H NMR features of antihistamines in the presence and absence of cyclodextrins

4.4 RS Dimethindene Maleate (20)

4.4.1 ^{1}H NMR features of dimethindene maleate



(20)

At 400 MHz, the spectrum for dimethindene maleate was fully resolved. In the low field region, all eight aromatic protons were resolved (Table 4.3). Four of these signals had chemical shifts, multiplicities and rank order typical of the protons of 2-ethylpyridine (Fig 4.7).

The doublet (7 Hz) near 7.4 ppm, placed between the H-3' and H-5' pyridyl signals and the doublet (7 Hz) at 6.9 ppm are assigned to H-4 and H-7 (not necessarily respectively) of the indene ring system. The closely paired doublet of triplets (dt), centred at 7.1 ppm, are assigned as H-5 and H-6 of this ring system. Firm evidence of these assignments is provided by the results of the 2D ^{1}H/^{1}H COSY 45 plot (Fig 4.9) which revealed ortho, meta, and para couplings - the last not being evident using the traditional 1-D spectrum.

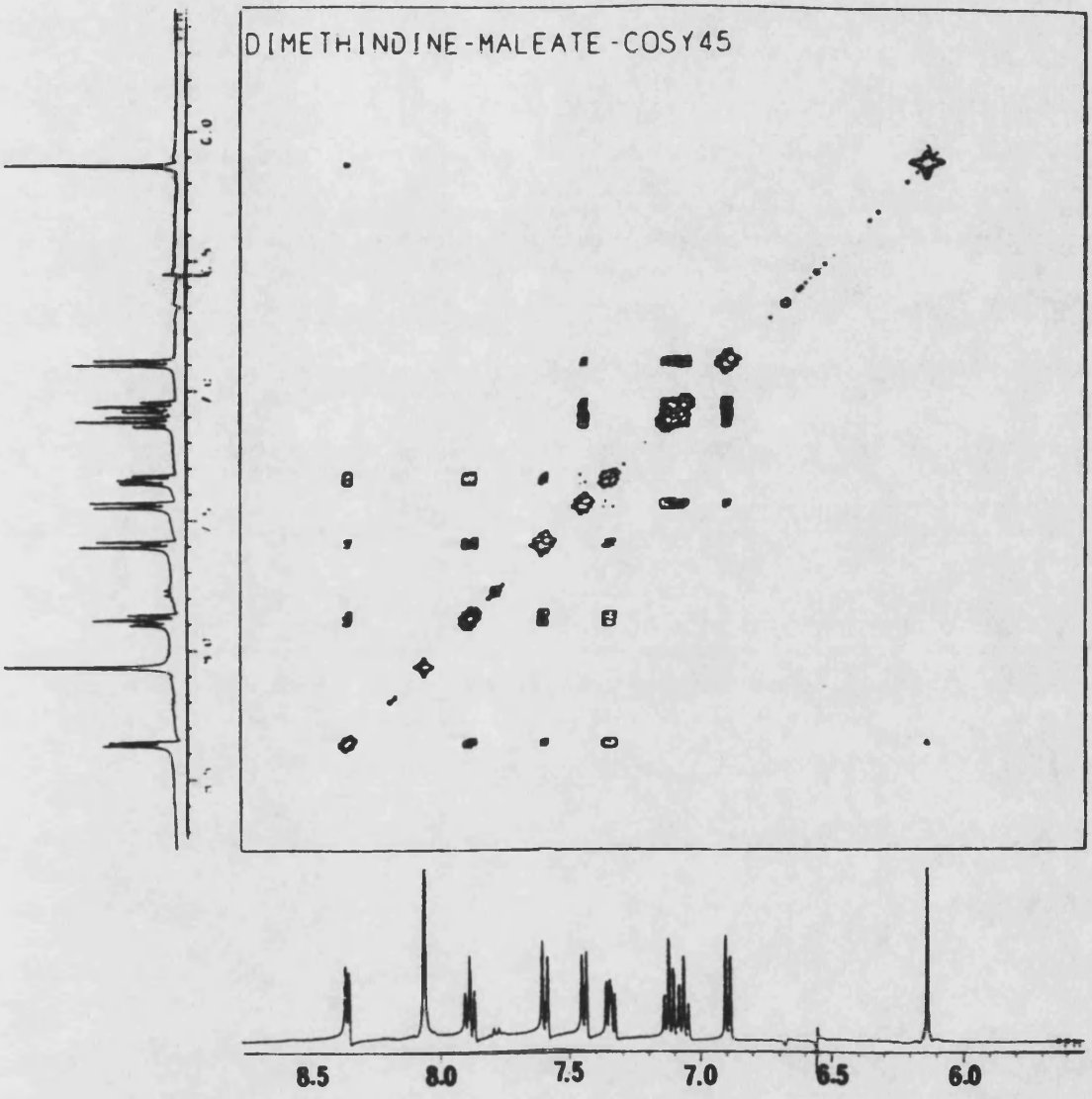


Fig 4.9 2D $^{1}H/^{1}H$ COSY 45 plot of dimethindene maleate in D_2O, run at 400 MHz

COSY 45

The COSY 45 ^{1}H-^{1}H NMR experiment produces a plot in which the identical 1-D spectra are displayed along the two axes. Corresponding resonances give rise to contour lines along the diagonal while coupled resonances produce lines offset from the diagonal (off diagonal or crosspeaks).

For example

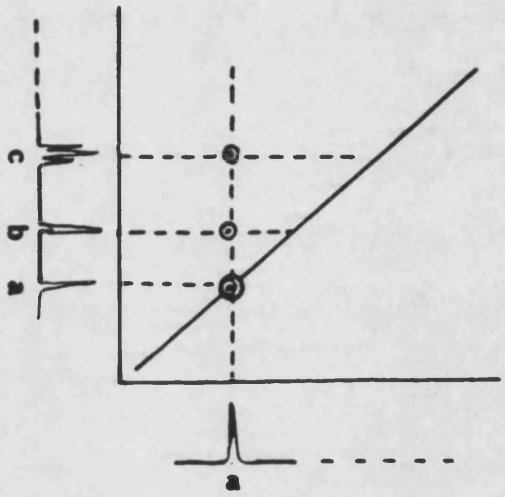


Fig 4.10

Thus if signal a is coupled to signals b and c, two offset lines will be seen parallel with signal a and one each for b and c (Benn and Gunther, 1983).

The results of the low field COSY plot for dimethindene maleate are shown in Table 4.4.

Table 4.4 Low field COSY couplings for dimethindene maleate in D_2O (at 400 MHz)

Resonance signal (proton)	Coupling interactions from COSY
6' (Py)	4', 3'[a], 5'
4' (Py)	6', 3', 5'
3' (Py)	6'[a], 4', 5'
7(4)	5/6, 4(7)[a]
5' (Py)	6', 4', 3'
5/6	7(4), 4(7)
4(7)	7(4)[a], 5/6

a indicates a very weak, (para) coupling

These results confirm that the doublets near 7.4 ppm is either H-4 or H-7, while the doublet immediately to low field (near 7.6 ppm) is a pyridyl proton (3'). The COSY experiment also clearly reveals the three coupling interactions of each pyridyl proton - these were not evident from the 1-D spectrum in the cases of H-3', H-5' and H-6'.

Assignments in the high field region (0-6.2 ppm) are as follows: The maleate protons give rise to a sharp singlet at 6.14 ppm. The quartet at 4.54 ppm (6.8 Hz) must be due to Me-CH of C-8, linking the indene to the pyridyl ring, whilst the doublet at 1.69 ppm (6.8 Hz) was assigned to the Me-CH of the same carbon. The broad singlet at 2.87 ppm, integrating for six protons, was assigned to the $N\text{-}Me_2$ group.

Table 4.3: 400 MHz ^{1}H NMR characteristics of RS dimethindene salts in D_2O in the absence and presence of 1 mole equivalent (approx.) of β-cyclodextrin[a]

Compound	Aromatic proton signals[f]							
	6'(py)[c]	4'(py)	3'(py)	7(4)	5'(py)	5,6	4(7)	Other signals
RS dimethindene (20) maleate[b] in D_2O	8.37 d(5)[d]	7.88 dt (8,8,1.5)	7.59 d(8.5)	7.44 d(7)	7.34 dd (5,7)	7.12 7.08 dt(7,1)	6.9 d(7)	8-H 4.54 q(7.5)
plus β-cyclodextrin	8.46 narrow t (5)[d]	7.93 tt(8,8, 1.5,1.5)	7.53 7,48 2d(8)	e	e	7.11 7.05 2t(7,7)	6.98 6.94 2d(7)	8-H 4.57 2q sep[n] 3Hz
RS dimethindene (20) fumarate[g] in D_2O	8.4 dd (7,1.5)	8.27 dt (10,10,2)	7.88 d(10)	7.37 d(9)	7.65 dd (7,7)[h]	7.05 6.97 2dt (9,9,1.5)	6.67 d(9)	8-H 4.65 q(6.8)
RS dimethindene (20) tartrate[i] in D_2O	8.46 dd (6,1.5)	8.26 dt (8,8,1.5)	7.9 d(8)	7.5 d(7)	7.68 dd (7,7)[h]	7.18 7.10 2t(7,7)	6.84 d(8)	8-H 4.7 q(7)
plus β-cyclodextrin	8.5 m(w 11)	8.3 centre 2dt (8,8,1.5)	7.88 d(7) 2d(8)	7.43 2dd	7.69 t(8,8) sep[n] 1Hz	7.10[j] 2d(9) 7.05 2t (8,8, sep[n] 4)	6.77	8-H unresolved overlaps tartrate signal

Footnotes - Table 4.3

a =chemical shifts in ppm from TMS, ; multiplet separations (Hz) in parentheses;
abbreviations s singlet, d doublet, t triplet, m multiplet, b broad,
b =6.14 ppm s
c =of pyridyl ring
d =each line shows fine structure 1Hz
e =m near 7.38 ppm
f =aromatic assignments confirmed by a COSY plot
g =6.37 ppm s
h =centre lines overlap
i =4.32 ppm s
j =probable assignment 5-H

The remaining signals at 3.42 ppm (s, 2H), 3.37 ppm (m, 1H) 3.27 ppm (m, 1H) and 2.96 ppm (m, 2H) are assigned to C-1 methylene and the ethylamino side chain. More specific assignments are not possible.

4.4.2 Spectral changes in the presence of β-CyD

Significant changes in the resonance signals of both the host (β-CyD) and guest (dimethindene) were seen when a 1:1 mixture (approximately) of the two components, in D_2O, was examined and compared with spectra of the two components in isolation (see Table 4.3).

Host Signals

Upfield shifts of the host molecule signals were seen for H-3, H-5 and $H_2$6 i.e. those due to the protons attached to the interior of the CyD cavity, while resonances of the external protons (H-1, H-5 and H-4) were little altered.

In the spectra of these complexes, the CyD signals were best resolved with (+)-dimethindene tartrate as the guest molecule (Fig 4.11). Under these conditions the upfield shifts (in ppm) of the CyD protons noted, were; H-1 nil, H-3 0.25, H-6_a6_b 0.07, H-5 ≃ 0.25, H-2 nil and H-4 0.05.

Fig 4.11 Expanded ^{1}H NMR spectrum of ß-CyD signals when complexed with dimethindene

Guest Signals

The pyridyl H-6', H-4' and H-3' and the highest field aromatic proton H-4(7) resonances all appeared in duplicate form, with those of H-3' and H-4(7) showing the greatest separation of the antipodal signals (Fig 4.12).

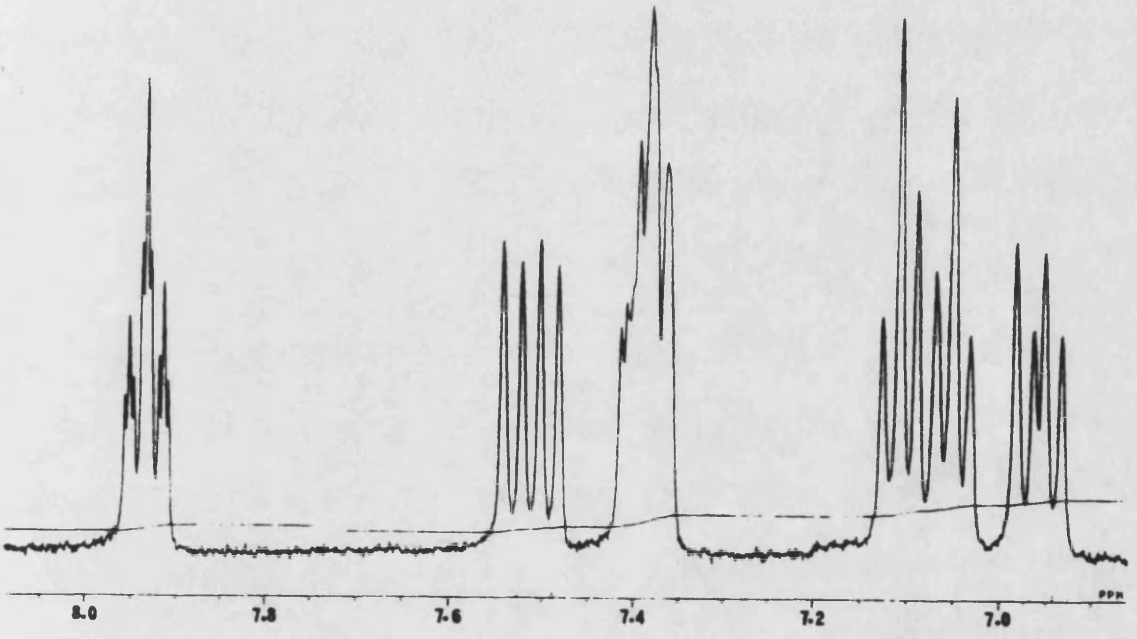


Fig 4.12 Expanded ^{1}H NMR spectrum of the low field region of dimethindene after complexation with β-CyD

Thus duplication of the H-4' (dt) leads to a triplet of triplets (tt) separated by ≈2 Hz at 400 MHz, while the H-3' doublet is converted to a pair of well resolved doublets (2d) of separation ≈ 16 Hz. The H-5' and H-7(4) signals converged together to form an unresolved multiplet. The H-5/6 triplets were little changed although some loss of fine structure was noted, while the H-4(7) resonance appeared as a pair of doublets of separation ≈7 Hz.

The only high field signal that was significantly affected was the $\underline{CH}$-CH_3 proton of C-8 which appeared as two quartets (4.58 ppm) of ≈3 Hz separation.

4.4.3 The nature of the complex

The stoichiometry of the complex can be obtained by recording the spectra of solutions, in D_2O, where the ratio of the two components i.e. host (β-CyD) and guest (dimethindene maleate), is varied, but where the overall molar concentration is constant. Table 4.5 shows the shifts for β-CyD: dimethindene maleate solutions of seven chosen molar ratios, at 400 MHz. Then, by performing a Job Plot (Nakajima et al, 1984) for, for example, H-3 of the CyD (Table 4.6, Fig 4.13), the curve maximizes at 0.5, indicating a 1:1 complex.

Table 4.6 Data for Job Plot of H-3 (β-CyD) of dimethindene maleate:β-CyD complex (calculated from 270MHz spectra)

[β-CyD]	[dim]	Δ Hz	$\frac{\Delta \times [\beta\text{-CyD}]}{[\beta\text{-CyD}]+[dim]}$	$\frac{[dim]}{[\beta\text{-CyD}]+[dim]}$
3/4	1/4	56	42	0.25
2/3	1/3	60	40	0.33
1/2	1/2	92	46	0.50
1/3	2/3	108	36	0.67
1/4	3/4	112	28	0.75

Similar plots were obtained for H-5 and the aromatic protons, H-6', H-5', H-4', H-3' and H-4(7) (see Fig 4.13).

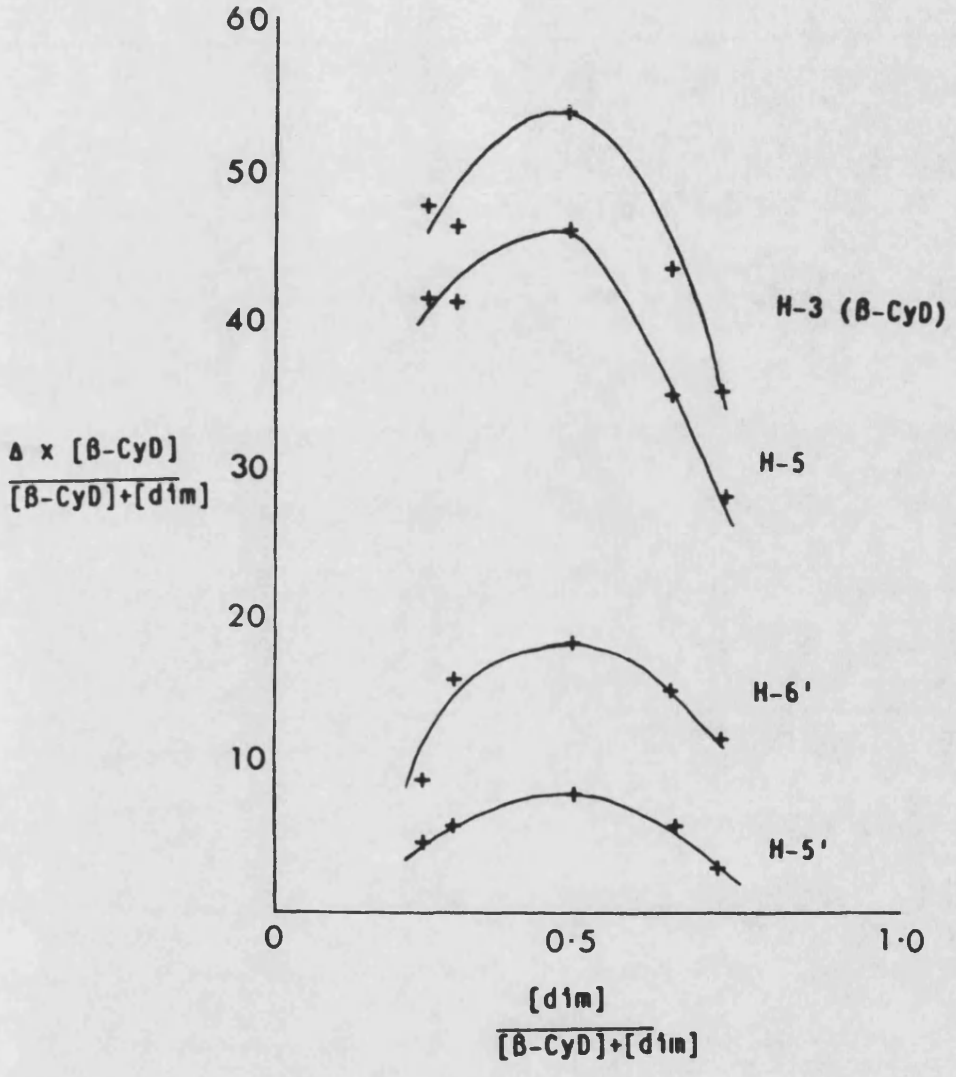


Fig 4.13 Job Plot for a variety of dimethindene and CyD protons when complexed with β-CyD

Table 4.5 Experimental data for constant variation (Job) plots of dimethindene maleate:β-CyD complex (270 MHz)

				Chemical Shifts (ppm)												
				β-CyD						aromatic dimethindene maleate						
Job	Plot	[β-CyD]:[dim]		H-1	2	3	4	5	6-H_2	6'	4'	3'	7(4)	5'	5/6	4(7)
1		1 :	0	-	-	-	-	-	-	8.37	7.88	7.61	7.45	7.36	7.12	6.91
2		3/4 :	1/4	4.98	3.62	3.66	3.49	2.50	3.74	8.40	7.91	7.58	7.45	7.37	7.09	6.93
3		2/3 :	1/3	4.99	3.62	3.67	3.51	3.53	3.77	8.43	7.92	7.57	7.43	7.38	7.09	6.95
4		1/2 :	1/2	5.00	3.62	3.71	3.53	3.58	3.79	8.46	7.93	7.54	7.40	7.40	7.10	6.97
5		1/3 :	2/3	5.02	3.62	3.79	3.53	3.68	3.81	8.48	7.94	7.50	7.40	7.40	7.10	7.00
6		1/4 :	3/4	5.02	3.63	3.80	3.53	3.69	3.82	8.49	7.94	7.50	7.41	7.41	7.12	7.00
7		0 :	1	5.05	3.62	3.94	3.56	3.85	3.85	-	-	-	-	-	-	-
		Hz		-	-	-	0.28	-	0.35	0.11	0.12	0.06	0.11	0.05	0.05	-	0.09

Hz is the difference between the free value and the complexed value

The spectra of the fumarate and tartrate salts of RS dimethindene were similar to that of the maleate except that the H-5' (Py) and H-7(4) resonances were interchanged (see Table 4.3). This gives us evidence of the unique shielding influence of the maleate anion in the ion pair. After addition of β-CyD to the tartrate salt, all the aromatic signals remained resolved and all except H-7(4) and H-5/6 were duplicated. The separations for the tartrate were H-6' (Py) ≈2 Hz, H-4' ≈3.5 Hz, H-3' 12 Hz, H-5' 12 Hz, H-5(6) ≈3 Hz and H-4(7) ≈8 Hz.

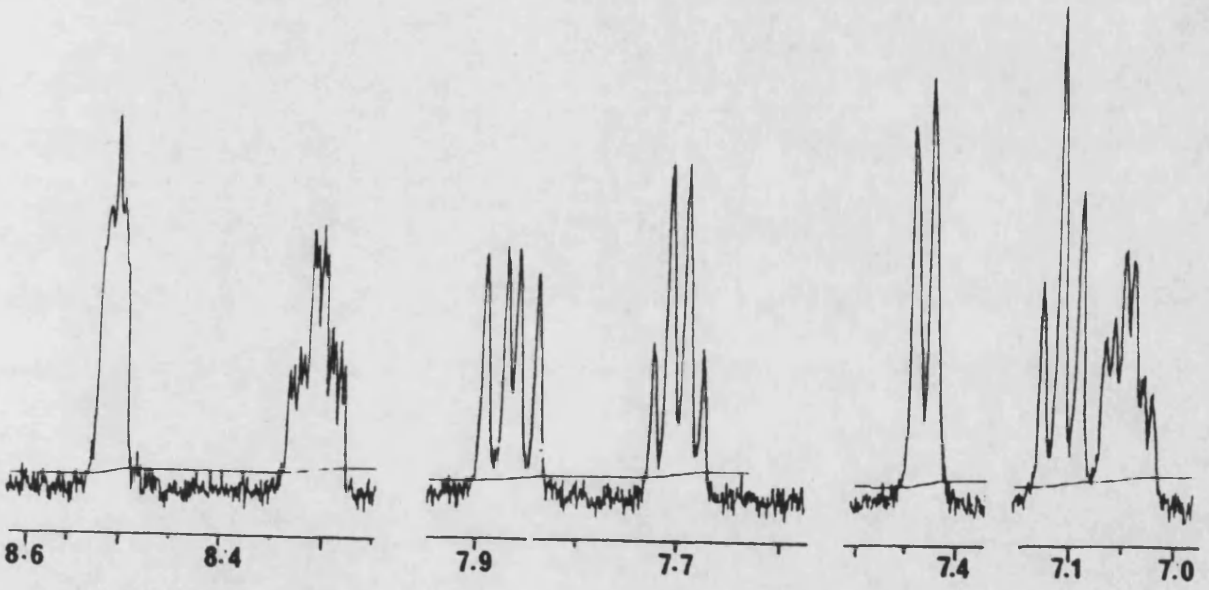


Fig 4.14 Expanded ^{1}H NMR spectrum of low field region of dimethindene (tartrate) after complexation with β-CyD

On the basis of extents of separation of the chiral resonances, rather than chemical shift differences following complexation - it is probable that the protonated dimethindene molecule enters the CyD torus with a carbon 5-4-8-3'-4'-5'-6' leading. (Fig 4.15)

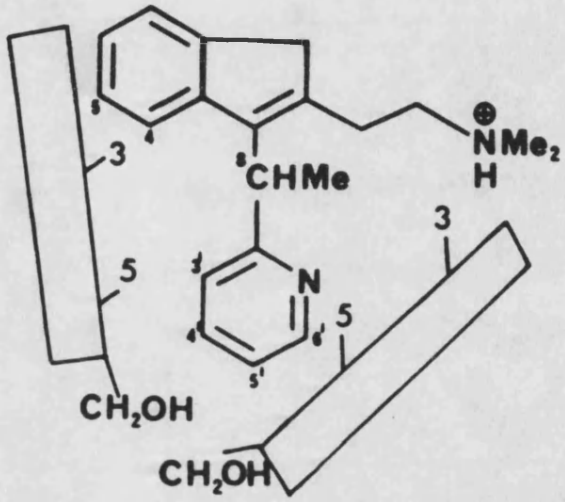


Fig 4.15 Cross section of CyD complex with the protonated dimethindene indicating the proposed position of the guest in the cavity

4.4.4 Effect of ring size

The spectra of RS dimethindene salts were little affected by changing the CyD to that of either α or γ. The separation of the chiral resonances were not as clear and although some changes were visible, the resolution was not as good as that with β-CyD.

4.4.5 Optical purity measurements

From the aspect of optical purity, the appearance of the H-3' (Py) resonance has the greatest potential since this occurs as a pair of resolved doublets of separation 16 Hz in the 400 MHz spectrum of the racemic maleate. The H-3' (Py) signals in the 270 MHz spectra of antipodal maleates (recrystallized to constant optical rotations) formed near-symmetrical doublets with no evidence of antipodal impurity i.e. no distortion of the slope of the lower field edge of the dextro signal, or higher field edge of the levo signal (Table 4.7).

Table 4.7 Doublet line chemical shift values (in ppm) for the H-3' proton of racemic, dextro and levo dimethindene maleate in the presence of β-CyD (270 MHz, D_2O).

	Chemical shift (ppm)			
RS	7.48	7.50	7.52	7.54
dextro	7.48	7.51	-	-
levo	-	-	7.52	7.55

Spiking experiments were performed by recording inclusion spectra of the dextro isomer (10.5 mg in 0.5 ml D_2O) mixed with increasing amounts of the levo isomer (2 µl, 5 µl, 10 µl, 20 µl and 50 µl of a 12.5 mg in 0.3 ml D_2O solution) and monitoring the appearance of the H-3' doublet. The limit of detection was found to be between 0.76 (2 µl levo isomer added) and 1.96% (5 µl levo isomer added) (Fig 4.16). On this basis resolved material contained no more than 1% of the minor isomeric form.

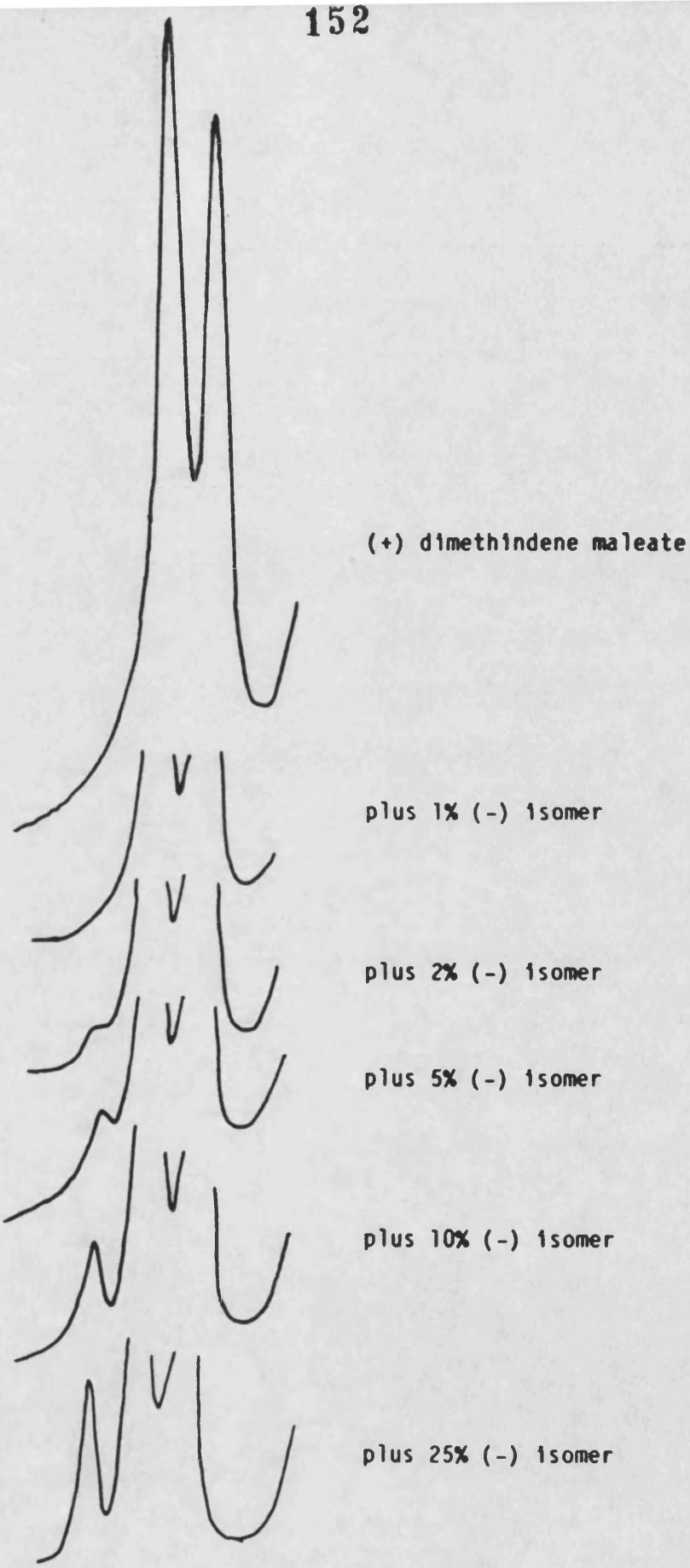
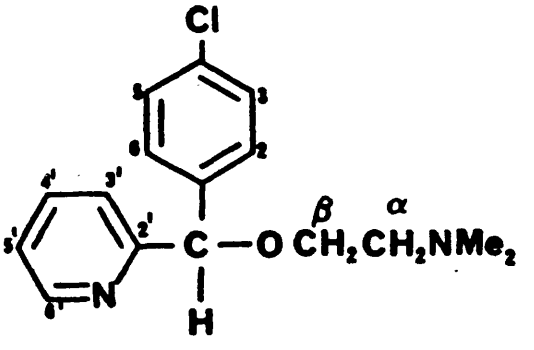


Fig 4.16 Expanded 270 MHz ^{1}H NMR spectrum of H-3' proton resonance signal showing change of dextro signal on addition of levo isomer.

4.5 RS Carbinoxamine Maleate (9)

4.5.1 ^{1}H NMR features of carbinoxamine maleate



(9)

In the low field region of the spectrum of the RS (9) maleate (400 MHz, D_2O) three of the eight aromatic proton signals were resolved (Table 4.8). Evidently these were the pyridyl protons from their chemical shifts and multiplicities i.e. H-6' dd near 8.44 ppm, H-4' dt centred at 7.8 ppm and H-3' as a doublet near 7.47 ppm. The H-5' (Py) and the four protons of the p-chlorophenyl ring gave a near singlet at 7.31 ppm.

In the high field region (0-6.2 ppm), the assignments are as follows. The two maleate protons produce a singlet at 6.17 ppm. The one proton singlet at 5.57 ppm must be due to the methine proton attached to the benzylic carbon that links the two aryl groups. The narrow triplet at 3.75 ppm and the multiplet at 3.38 ppm, both integrating as two protons, are assigned as the $O\underline{CH_2}$ (β) and $\underline{CH_2}N$ (α) protons respectively. The $N\underline{Me_2}$ signal formed two closely placed broad singlets near 2.83 ppm, showing evidence of non-equivalent NMe_2 groups.

4.5.2 Spectral changes on addition of β-CyD

Addition of β-CyD to a solution of RS carbinoxamine maleate, in D_2O, resulted in duplication of the pyridyl H-4' (sep^n 2.5 Hz), benzylic CH (sep^n 3.2 Hz) and one of the non-equivalent N-methyl resonances. The pyridyl H-6' signal was little changed while H-3' and the rest of the aromatic signals (doublet and apparent singlet before inclusion) overlapped to form a complex multiplet (Table 4.8)

In the spectrum of the RS tartrate (Table 4.8), all the aromatic resonances were resolved, but again after inclusion into the β-CyD, only the pyridyl H-6' and H-4' signals (the latter duplicated) remained isolated. Duplication of the high field N-methyl resonance was clear as was also that of the benzylic signal.

4.5.3 Effect of ring size

Full resolution of the aromatic resonances was also achieved when the RS maleate was included in γ-CyD and all except the H-6' (Py) signal were duplicated (Table 4.8). Remarkably the N-methyl resonance appeared as a broad 6-proton singlet. The benzylic signal was also duplicated and chemical shift differences of 6.6 Hz, at 270 MHz, and 9.8 Hz, at 400 MHz, were greater than that observed with β-CyD at 400 MHz (3.2 Hz). This signal was therefore chosen for optical purity assessments.

On the basis of separation of signals after inclusion i.e. benzylic CH, 10 Hz: H-4', 2 Hz: H-3', 4 Hz: H2/6, 3.5 Hz: H3/5, 2 Hz: it is proposed that the protonated carbinoxamine molecule enters the cavity as shown, Fig 4.17.

Table 4.8: 400 MHz ^{1}NMR characteristics of RS carbinoxamine salts in D_2O in the presence and absence of 1 mole equivalent (approx.) of cyclodextrin[a]

Compound	Aromatic proton signals 6'(py)[c]	4'(py)	3'(py)	5'(py)	2,3,5,6(A_2B_2) of 4-ClC_6H_4	Other signals
RS carbinoxamine (9) maleate[b] in D_2O	8.40 d(5)[d]	7.81 t (8,8,1.5)	7.47 d(8)	e	e	benzylic CH 5.57 s NMe_2 2.85, 2.82 bs
plus β-cyclodextrin	8.52 d(4.5)	7.91 2dt (8,8,1.5, sep^n 2.5)	f	f	f,g	benzylic CH 5.626, 5.618, 2s sep^n 3.2Hz NMe_2 2.90 bs 2.86 b signal resolved into 2 lines at peak
plus γ-cyclodextrin	8.5 d(4.5)	7.9 tt (7.2,7.2, 1.5) sep^n 2Hz	7.45 2xd (7.2) sep^n 4Hz	7.39 dd (5.5,7) sep^n 1.5Hz	h	benzylic CH 5.565, 5.541, 2s sep^n 10Hz NMe_2 s 2.87
RS carbinoxamine (9) tartrate[i] in D_2O	8.54 d(5)[d]	8.05 dt (8,8,1.5)	7.62 d(8)	7.55 ddd (6,5.5, 1.5)	7.38 centre of A_2B_2 signal[j]	benzylic CH 5.75 s NMe_2 2.89 s 2.83 s
plus β-cyclodextrin	8.59 d(4.5)[d]	8.03 tt (8,8,1.5)		k	k,l	benzylic CH 5.69 bs NMe_2 2.88 s 2.85, 2.84 2s

Footnotes - Table 4.8

a =chemical shifts in ppm from TMS ; multiplet separations (Hz) in parentheses;
abbreviations s singlet, d doublet, t triplet, m multiplet, b broad.
b = 6.14 ppm s
c = of pyridyl ring
d = each line shows fine structure 1Hz
e = overlaps 4-Cl C_6 H_4 signal to give apparent s at 7.31 ppm, of integral 5.
f = overlap to form multiplet 7.3-7.5 ppm
g = A_2B_2 signal resolved: A_2 2d (8.3) sep^n 4Hz 7.43 ppm
B_2 2d (8.3) sep^n 3Hz 7.36 ppm
h = A_2B_2 signal resolved: A_2 2d (9.0) sep^n 3.5Hz 7.24 ppm
B_2 2d (9.0) sep^n 2Hz 7.00 ppm
i = 4.32 ppm s
j = outer lines of low intensity
k = overlap to form 7.35-7.55 ppm m
l = A_2B_2 signal resolved: A_2 2d (8.3) sep^n 4.5Hz 7.48 ppm
B_2 2d (8.3) sep^n 3Hz 7.41 ppm

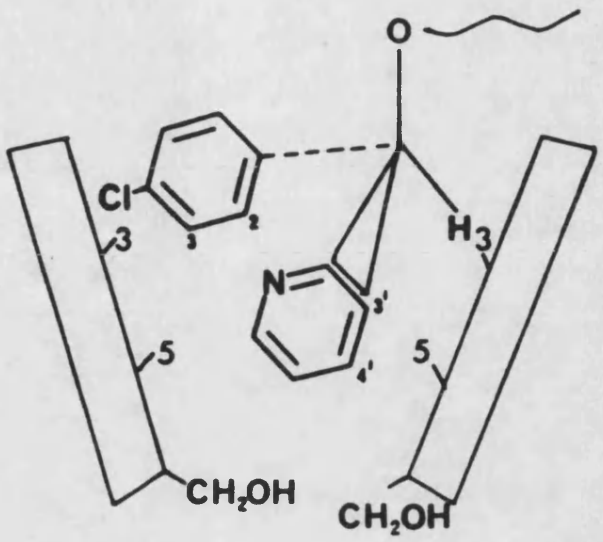


Fig 4.17 Cross section of CyD cavity indicating the proposed position of the protonated RS carbinoxamine

4.5.4 Assessment of optical purity

In the 400 MHz inclusion spectra (γ-CyD) of samples resolved for this work (2.7.3) antipodal benzylic CH resonances both formed sharp singlets, (+)carbinoxamine 5.61 ppm and (-)carbinoxamine 5.63 ppm, which merged smoothly with base line noise and showed little evidence of antipodal impurity. Spiking experiments, carried out (as described for dimethindene) showed that 1% of the minor isomer could be detected by examination of the CH resonance. The methine resonances of the antipodal carbinoxamine samples obtained from McNeill Laboratories clearly revealed these to be incompletely resolved (≈ 3% impurity) (Fig 4.18).

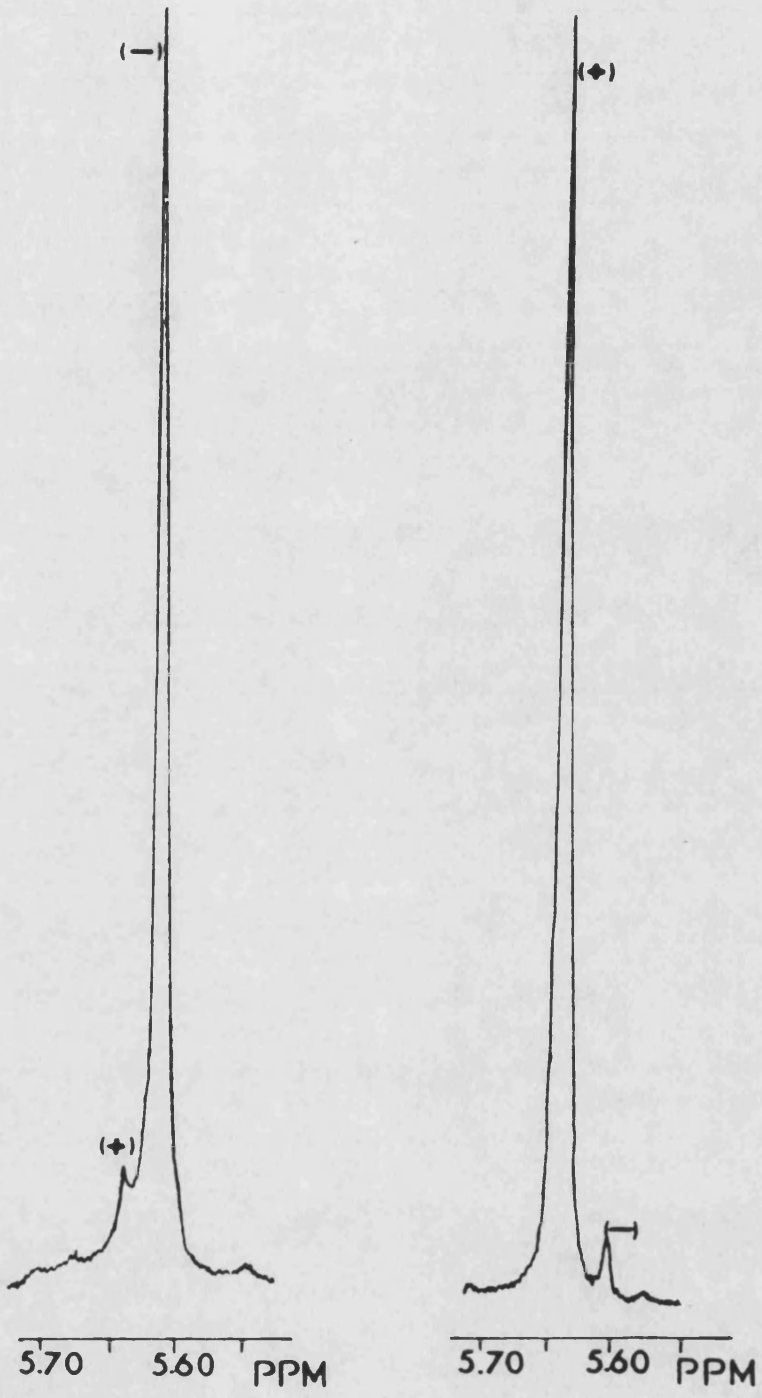
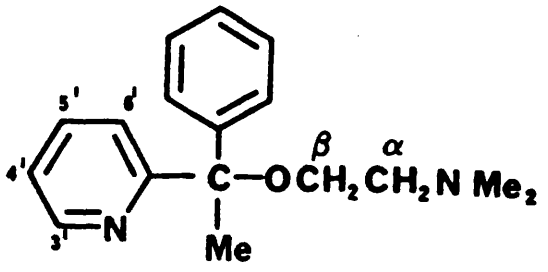


Fig 4.18 270MHz ^{1}H NMR benzylic proton signals of incompletely resolved enantiomers of carbinoxamine tartrate in D_2O complexed with γ CyD.

4.6 RS Doxylamine Succinate



(10)

In the low field region of the spectrum (400 MHz, D_2O) all the pyridyl resonances were resolved and could be identified as usual (Table 4.9). The five phenyl protons formed a multiplet centred at 7.4 ppm.

At the high field region, the multiplets centred near 3.68 ppm (1 proton, 8 line signal), 3.52 ppm (1 proton, 8 line signal) and 3.35 ppm (2 protons) were assigned as the α and β methylene signals.

The NMe_2 signal was composed of two broad singlets at 2.87 and 2.81 ppm of separation 25 Hz - another indication of the non-equivalence of the NMe_2 groups (cf NMe_2 of carbinoxamine maleate). The four proton singlet at 2.46 ppm was due to the succinate protons and the three proton singlet at 1.98 ppm to the methyl attached to the benzylic carbon.

Table 4.9: 400 MHz ^{1}H NMR characteristis of Rs doxylamine succinate in D_2O in the presence and absence of 1 mole equivalent (approx) of cyclodextrin[a]

Compound	Aromatic Proton Signals					Other Signals
	6'(Py)[c]	4'(Py)	3'(Py)	5'(Py)	2,3,4,5,6 of phenyl	
RS doxylamine(10) succinate[b] in D_2O	8.45 d(5)[d]	7.92 dt (8,8,1,5)	7.6 d(8)[d]	7.43 ddd (7.5,6,1.5)	7.39 m	NMe_2 2.87 bs 2.81 bs C-Me 1.98 s
plus β-cyclodextrin	8.6 d(5)[d]	7.88 centre 2dt(8,8,i.5 sep[n] 13.5)	7.43[f] centre 2d[g](8, sep[n]8	7.5 centre 2ddd(as above, sep[n],7)	7.2-7.35 m	NMe_2 2.83 bs C-Me 2.19 s

Footnotes - Table 4.9

a =chemical shifts in ppm from TMS ; multiplet separations (Hz) in parentheses; abbreviations s singlet, d doublet, t triplet, m multiplet, b broad.
b = 2.46 ppm singlet
c = of pyridyl
d = each line shows fine structure 1 Hz
e = five phenyl protons
f = note relative chemical shifts of 3'(Py) and 5'(Py) signals reversed in presence of β-CyD
g = form apparent triplet

In the presence of β-CyD (Table 4.9) the pyridyl H-4', H-3' and H-5' were all duplicated. H-4' changed from a dt to 2 dt of separation 13.5 Hz, H-3' to 2 d (giving an apparent triplet) of separation 8 Hz and H-5' gave a multiplet of separation 7 Hz (the signal width of this H-5' resonance actually increased by 6 Hz).

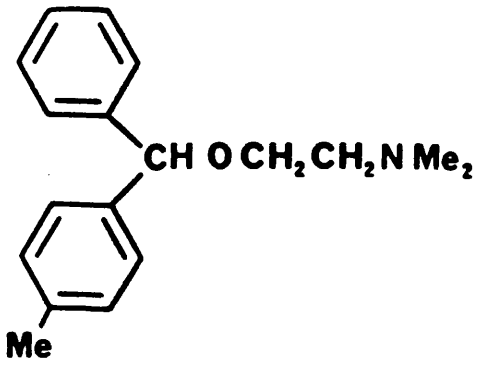
These results are notable in that the separation of the antipodal H-4' resonance was much greater than that observed for H-4' of dimethindene (2 Hz) and carbinoxamine (2.5 Hz). Separation of the H-3' and H-5' resonances were less than that of H-4' but still substantial. Also the relative chemical shifts of the H-3' and H-5' pyridyl protons were reversed on complexation with CyD.

The phenyl aromatic proton resonances increased in width and complexity on inclusion.

In the high field region, the two NMe resonances of the free molecule coalesced to form a broad singlet (2.83 ppm) after complexation, while the succinate (s, 2.48 ppm) and benzylic C-Me (s, 2.19 ppm) remained as singlets, the latter being shifted downfield by 0.21 ppm.

These results suggest a different mode of entry of the protonated doxylamine molecule into the cavity - possibly due to the methyl group attached to the benzylic carbon. In the case of carbinoxamine a large separation was seen for the benzylic CH - but in the case of doxylamine, very little change was visible for the benzylic methyl.

4.7 RS Neobenodine Hydrochloride



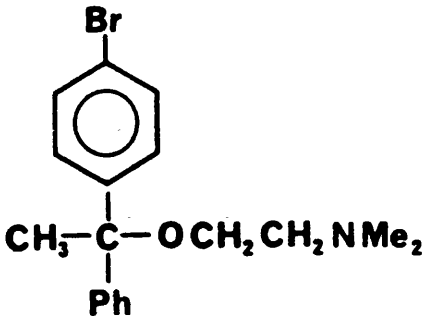
(8)

This compound is a non-pyridyl analogue of carbinoxamine. In contrast to carbinoxamine, its spectral features showed no dramatic changes after inclusion into the ß-Cyd - notably the benzylic C-H resonance remained unsplit.

The aromatic resonance was complex but the A_2B_2 four line signal of the p-tolyl unit was resolved. After complexation, both halves of the signal were duplicated with separation of about 1.25 Hz (lower field half) and 7.5 Hz (high field half).

No NMe non-equivalence was seen - evidence that its appearance in previous examples is dependent on the presence of the 2-pyridyl substituent. The sugar resonances gave evidence of complex formation (as described 4.4.2).

4.8 RS Mebrophenhydramine Hydrochloride



(11)

This compound is a non-pyridyl analogue of doxylamine, and once again the splitting of the NMe_2 resonance due to the non-equivalence of the two methyl groups was not seen.

At 400 MHz, in D_2O, the nine proton aromatic signal was composed of two halves of the p-bromo-phenyl A_2B_2 resonance which surrounded a multiplet due to the phenyl protons. In the high field region the α and β CH_2 protons gave rise to triplets at 3.47 and 3.25 ppm respectively. The resonance of the two equivalent methyl groups of the NMe_2 substituent gave a six proton singlet at 2.81 ppm and the C-Me group was visible as a three proton singlet at 1.77 ppm.

After inclusion into the CyD (β) cavity, the aromatic signal became more complex. The upper field half of the A_2B_2 signal (7.28 ppm) remained resolved and was duplicated into two doublets of separation 3 Hz.

Fig 4.19 illustrates the optical purity potential of this high field portion of the A_2B_2 signal.

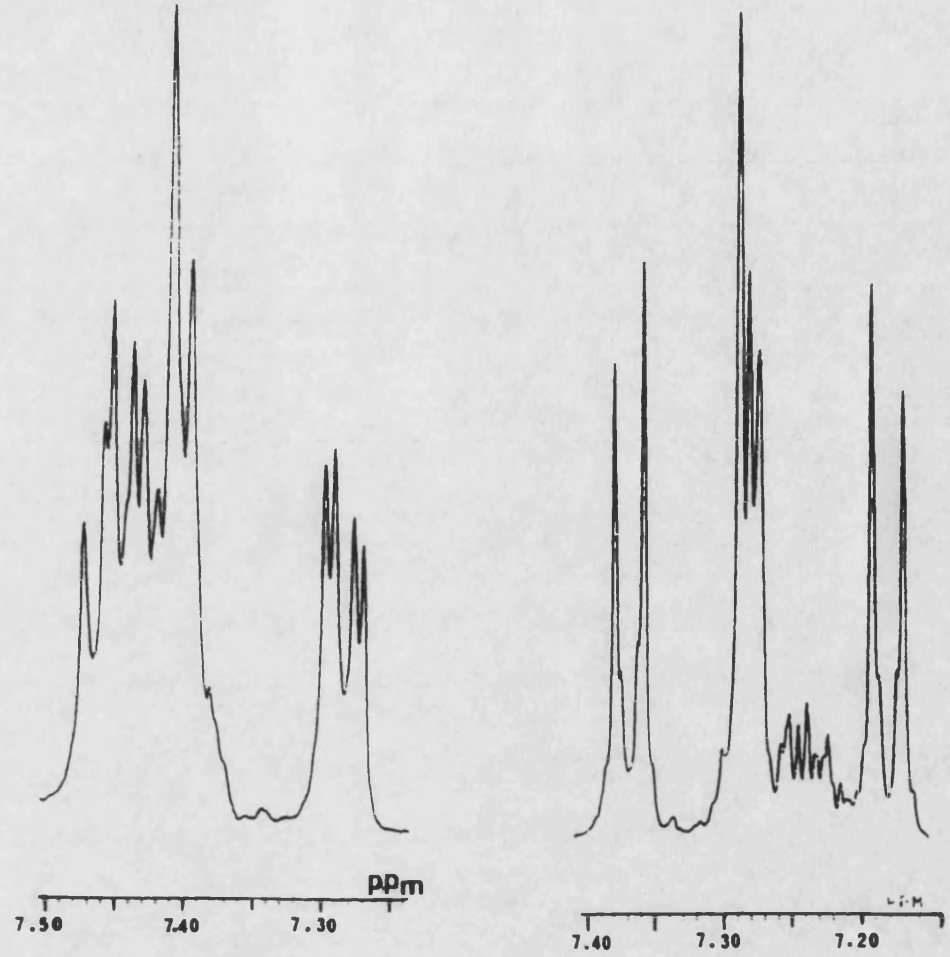
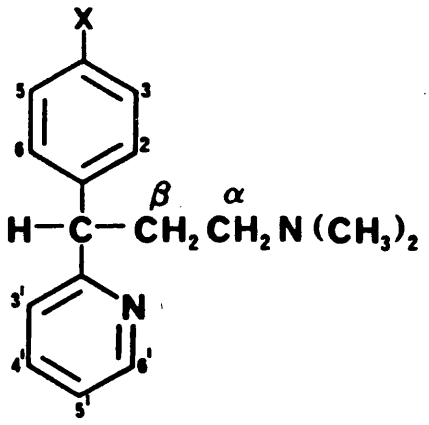


Fig 4.19 400 MHz ^{1}H NMR spectrum of high field portion of A_2B_2 signal of mebrophenhydramine maleate in D_2O in the presence and absence of β-CyD

The signal due to the RS maleate mixture showed two overlapping doublets, while that of a resolved sample i.e. dextro isomer maleate (details of the resolution 2.7.4) showed a sharp doublet with no evidence of the other (levo) antipodal signal. The high field signals, of the HCl salt, showed little change; the NMe_2 signal was unchanged and the C-Me resonance broadened. In the 400 MHz inclusion spectra of the RS and (+)maleate, however, the NMe_2 resonance split into two singlets of a few Hz separation (2.7 Hz) - indicating again some conformational change but no chiral differences.

4.9 The next three examples concern the trio



Where X = H, pheniramine (17), X = Cl, chlorpheniramine (17c), and X = Br, brompheniramine (17b).

RS Pheniramine maleate (17)

In the low field region (400 MHz, D_2O), the pyridyl protons H-6', H-4' and H-3' could be assigned as previously described. The H-5' signal overlaps with the phenyl proton resonances. In the 0-6.1 ppm region, the two maleate protons were visible as a singlet at 6.07 ppm and the benzylic CH signal as a triplet at 4.07 ppm (Table 4.10). The non-equivalence of the two NMe_2 groups were not apparent and this signal was therefore seen as a singlet. The 9 line multiplet at 2.92 ppm and the multiplet (in two halves) at 2.44 ppm were assigned as the $C\underline{CH_2}$ (β) and $\underline{CH_2}N$ (α) protons respectively.

Addition of β-CyD to a solution of RS pheniramine maleate in D_2O, resulted in duplication of the pyridyl signals H-6' (sep^n 2.5 Hz), H-4' (sep^n 1.5 Hz) and H-3' (sep^n 5 Hz). The H-5' and the phenyl resonances overlapped to give an unresolved multiplet.

In the high field region the benzylic CH signal duplicated to become a four line signal of separation 3 Hz. The α and β methylene signals both shifted downfield, the β proton resonances splitting the two halves of the signal into two eight line multiplets, centred at 2.77 and 2.50 ppm and the α proton resonance splitting into two six line signals a 3.2 and 3.1 ppm.

The sugar signals also showed evidence of complexation, as previously described (4.4.2).

RS chlorpheniramine maleate (17c)

In a spectrum run at 400 MHz in D_2O, the pyridyl H-6', H-4' and H-3' signals were resolved and identified as usual (Table 4.10). The A_2B_2 resonance of the p-Cl-phenyl unit was resolved but its low field half overlapped with the H-5' pyridyl signal.

In the high field region the triplet at 4.17 ppm (8 Hz) was assigned as the benzylic CH, while the triplet at 3.01 ppm (8 Hz) and the two multiplets at 2.55 and 2.47 ppm were assigned as the α and β CH_2 protons. The NMe_2 group was again seen as a singlet at 2.81 ppm - indicating the equivalence of all the protons.

Table 4.10 ^{1}H NMR characteristics of RS pheniramine maleate and its analogues, in D_2O, in the absence and presence of 1 mole equivalent of β-cyclodextrin[a]

Compound	Aromatic Proton Signals (Chemical shifts in ppm) H-6'(Py)	4'(Py)	3'(Py)	5'(Py)	Phenyl ring	Benzylic CH
RS pheniramine maleate[b] (17)	8.29 bd(5)[c]	7.65 bt(7.15, 1.5)	7.28 d(8)	d	d	4.07 t (8)
plus β-cyclodextrin	8.60 2xd sep^n 2.5	7.90 tt(8, 1.5) sep^n 1.5	7.44 dd(8.8) sep^n 5	e	e	4.27 4-line signal sep^n 3
RS chlorpheniramine maleate[b] (17c)	8.40 d(5)[c]	7.77 dt(8, 1.5)	7.37 d(8)	f	g	4.17 t (8)
plus β-cyclodextrin	8.50 b(2d)[h] sep^n 3	7.85 btt sep^n 3	i	i	i	bt 4.19
RS brompheniramine maleate[b] (17b)	8.3 d(5)[c]	7.57 dt(8, 1.5)	7.18 d(8)	7.12 dd(8, 1.0)	A_2B_2 2d 2.71 & 7.03	4.02 t (8)
plus β-cyclodextrin	8.57 2 d sep^n 2.5	7.90 dt sep^n 3	j	j	2 x dd 7.5(8) sep^n 4 7.35(8) sep^n 6	4.24 (duplicated but complex)

Footnotes - Table 4.10

a =chemical shifts in ppm from TMS ; multiplet separations (Hz) in parentheses;
abbreviations s singlet, d doublet, t triplet, m multiplet, b broad.

b 6.14 ppm s

c each line shows fine structure 1 Hz

d overlaps phenyl signal to give m 7.2 ppm (integral 6)

e overlaps phenyl signal to give an unresolved m at 7.35 ppm

f overlaps phenyl signal 7.29 ppm

g A_2B_2 signal resolved 2 d at 7.23 and 7.29 ppm

h visible as broad t signal

i overlaps to give a m with two major singlet at 7.34 and 7.35 ppm

j overlap with high field proton of duplicated A_2B_2 system

After addition of β-CyD to a solution of RS chlorpheniramine maleate in D_2O, duplications of the H-6' and H-4' resonances were visible. The other aromatic signals fused together to form a multiplet, but with two major singlets. In the case of the spectrum of dextro chlorpheniramine maleate and β-CyD - only one of these singlets was visible. Fig 4.20 indicates the potential of this signal in terms of optical purity assessment.

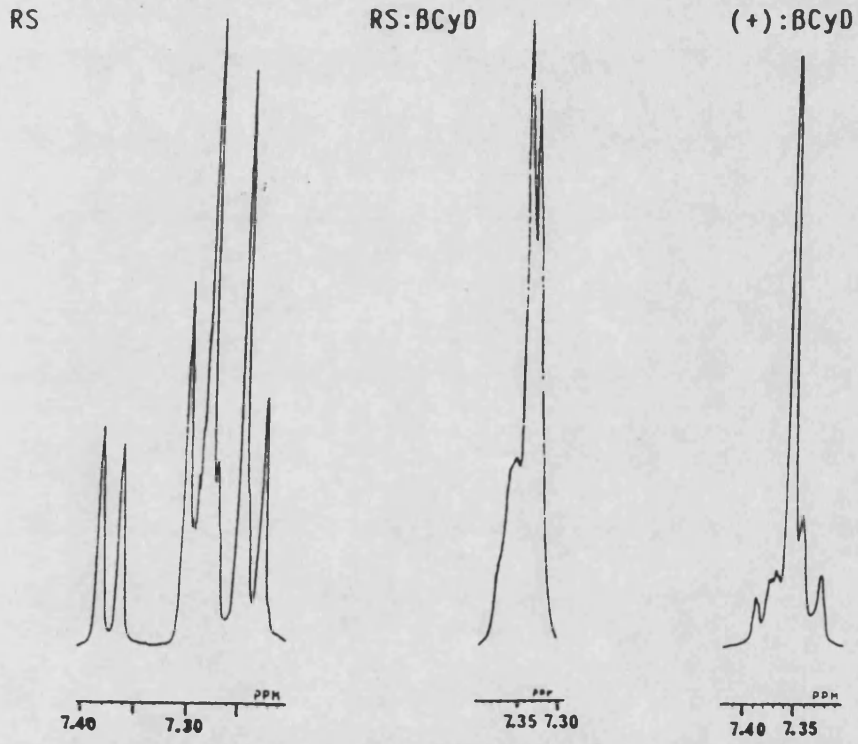


Fig 4.20 Expanded 270MHz ^1H NMR spectrum of chlorpheniramine maleate signals

This aromatic spectral feature of a resolved sample of (+)chlorpheniramine maleate with β-CyD indicated a high degree of optical purity (although not quantified).

The α CH_2 and β CH_2 protons changed as for pheniramine and the methylene CH triplet became a broad dt with a very small separation.

RS Brompheniramine maleate (17b)

At 400 MHz, in D_2O, the pyridyl signals could again be assigned by their chemical shift and multiplicity. The H-3' and H-5' signals were positioned in between the A_2B_2 signal of the p-Br phenyl resonance (Table 4.10). Patterns for the signals of the other proton groups were similar to those of pheniramine and chlorpheniramine i.e. benzylic CH, t at 4.02 ppm, the NMe_2 resonance was again a s at 2.74 ppm and the α and β CH_2 groups gave a triplet (2.93 ppm) and a two halved multiplet (2.35 and 2.48 ppm) respectively.

After inclusion in the CyD cavity the H-6' (sep^n 2.5 Hz) and H-4' (sep^n 3 Hz) pyridyl resonances were duplicated as for the other pheniramines. The H-3' and H-5' signals overlapped with the high field portion of the duplicated A_2B_2 resonance. Both protons of the A_2B_2 signal were duplicated to give a 2 dd (Table 4.10). The benzylic CH proton signal was also duplicated and the methylene CH_2 signals showed the same pattern as the other pheniramines. This splitting of the α and β CH_2 groups was also seen when (+)brompheniramine maleate was mixed with β-CyD and is therefore not due to chiral differences of the inclusion complex - but is probably due to a conformational change within the molecule upon inclusion into the CyD cavity.

Using the phenyl A_2B_2 signals as the best resonance duplication, some spiking experiments were carried out (as described for dimethindene) to measure the optical purity of the resolved samples (obtained from Schering). Using a solution of (+)brompheniramine and β-CyD and spiking with the (-)isomer - no change of the signal was evident on addition of 1 or 2% of the minor isomer. No further experiments were recorded since the sensitivity of the signal of choice proved to be poor.

4.10 Chiral additives for optical purity assessment in NMR

The technique of using a chiral acid in the resolution of racemic bases has been attempted by chromatographic techniques (Chapter 3) and should be transferable to an NMR experiment. The formation of diastereomeric ion pairs should result in differing chemical shifts of antipodal resonances.

Trials with RS chlorpheniramine base and a variety of chiral acids were carried out. The three acids used were d-10 camphorsulphonic acid (d-10 CSA), phenylsuccinic acid (PSA), and di-p-toluoyltartaric acid (di-pTTA) - acids that had been used in attempts to resolve chlorpheniramine by chromatographic methods.

Attempts using d-10 CSA and PSA were not successful and no duplication of any signal was seen. The use of di-pTTA (270 MHz, $CDCl_3$), however did prove successful. The best separation was seen for the benzylic CH triplet (7 Hz) near 4.1 ppm. In the absence of di-pTTA or using pure isomeric di-p-toluoyltartaric salt (obtained from resolution experiments) only a three line signal was seen. Using RS chlorpheniramine base and adding either (+) or (-) di-pTTA to the solution in $CDCl_3$ gave a six line signal of separation 2.7 to 5.4 Hz. Identification of each of the triplet lines could be easily achieved (Table 4.11, Fig 4.21).

Table 4.11 Chemical shifts of benzylic protons (triplet), at 270 MHz

	High Field Lines 1	1'	Centre Lines 2	2'	Low Field Lines 3	3'
RS Chlorpheniramine (-)di-pTTA	4.05	4.06	4.07	4.09	4.10	4.11
(-)Chlorpheniramine (+)di-pTTA	4.08		4.11		4.14	
(+)Chlorpheniramine (-)di-pTTA		4.12		4.15		4.18

A similar result was obtained using RS brompheniramine with di-pTTA. This would be a useful signal for the assessment of optical purity.

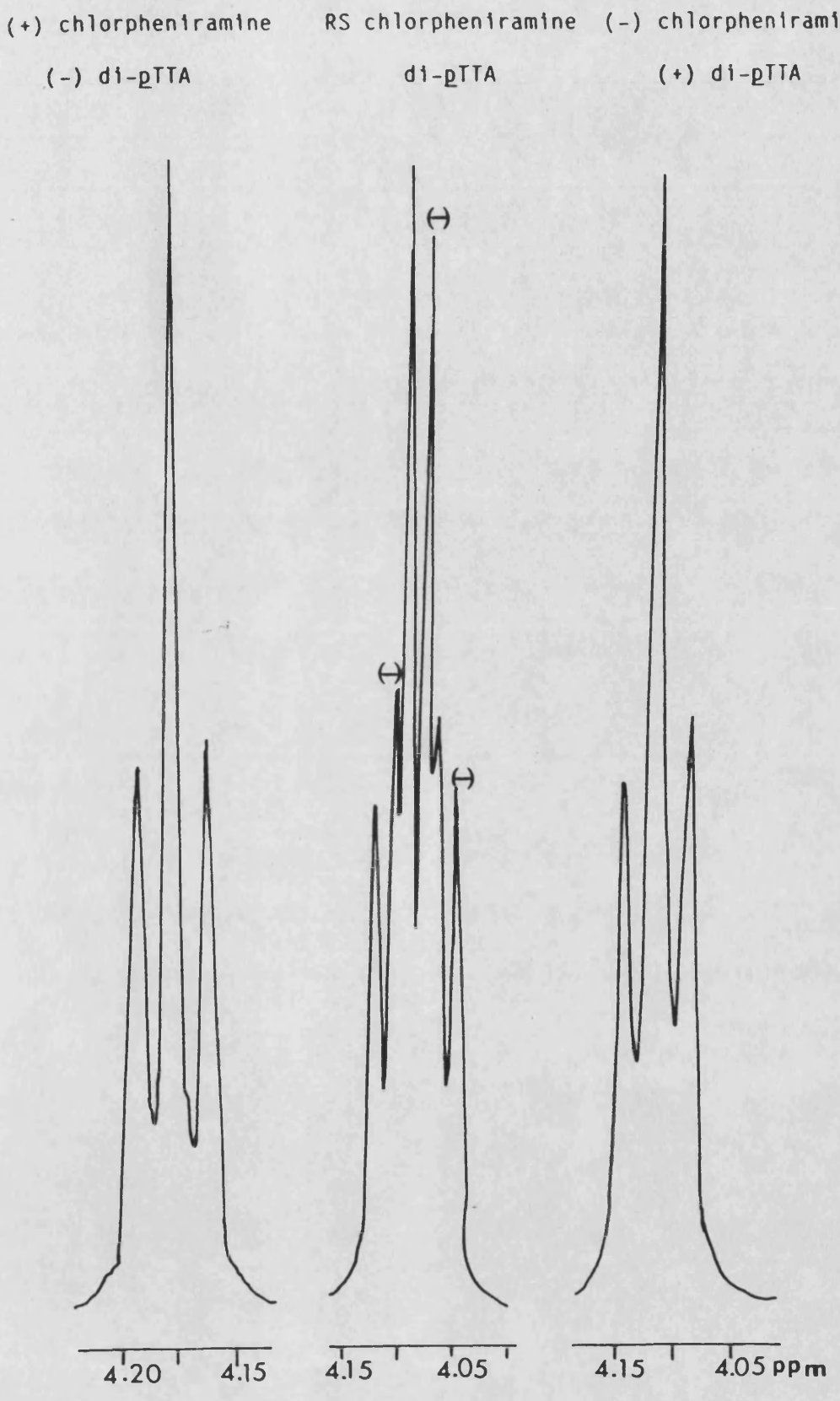
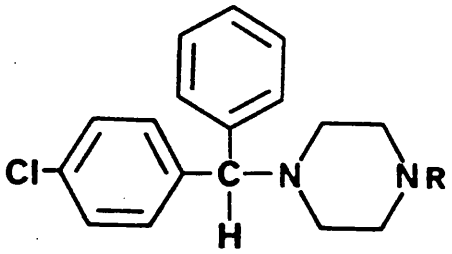


Fig 4.21 270MHz ^{1}H NMR resonance of the benzylic protons of RS chlorpheniramine maleate in the presence of di-pTTA

4.11 The next few examples involve the following type of compounds.



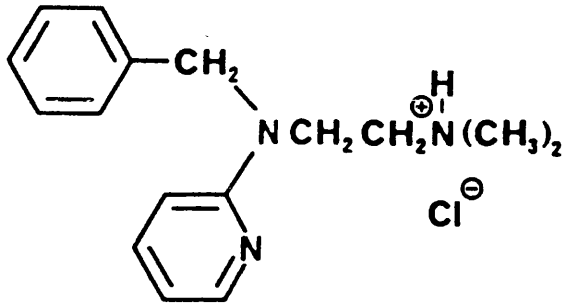
R = $CH_2C_6H_4$2-Me meclozine (13)

R = $CH_2CH_2OCH_2CH_2OH$ hydroxyzine (15)

Both of these compounds showed very little change in their spectral characteristics after complexation with β-CyD. The benzylic CH and the aliphatic side chain resonances were concealed by the CyD proton signals.

RS meclozine di HCl (13) showed a complex aromatic signal which became more complex on inclusion into the CyD cavity. In the case of hydroxyzine (di HCl) (15), the aromatic signals were again complex but on addition of β-CyD, duplication of the lower field half of the p Cl-phenyl A_2B_2 signal was visible.

4.12 The following achiral compound, tripelenamine hydrochloride (30).



(30)

was our only example of a compound showing no spectral changes after the addition of β-CyD. Similarly the sugar signals showed no evidence of complexation.

Chapter 5

Synthesis and Characterisation of Triprolidine and some of its analogues

5.1 Introduction

In addition to the study of antipodal pairs of chiral antihistamines, it was decided to include pairs of geometric isomers into this study. The classical example is that of triprolidine (23) and its Z-isomer (31).

It is of interest to obtain further data on this class and also to study the effect of various structural modifications on the antihistaminic activity of triprolidine such as replacement of Ar-4-Me by Ar-4-Et and 2-pyridyl by 3- and 4-pyridyl.

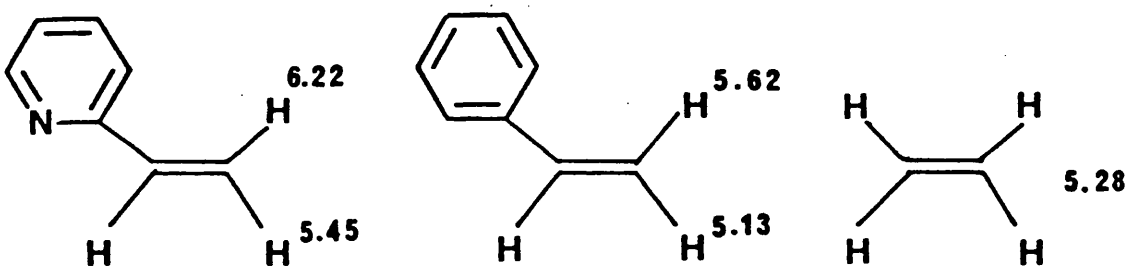
5.2 2-Pyridyl analogues

Samples of triprolidine (23) and its Z-isomer (31) were obtained from Burroughs-Wellcome (B-W) and were examined by UV spectroscopy and high field ^{1}H-NMR.

Evidence for the configuration of geometric isomers of this type was initially made from observation of their UV spectra in ethanol, using the method of Adamson et al (1957). In the case of triprolidine, the E-isomer (23) exhibited two absorption maxima at 229 and 276 nm to produce a UV spectrum very similar to that of 2-vinylpyridine (32), whereas the Z-isomer (31) exhibited a single maximum at 258 nm, typical of styrene (33). Adamson et al (1957) obtained comparable UV parameters for these compounds and interpreted their results according to the relative spatial arrangements of the aromatic functions about the olefinic bond. In the E-isomer the Ar ring is twisted out of plane of the double bond, (thus reducing the chromophoric effect of the styrenoid portion of the molecule) to avoid non-bonded interaction between the methylene amino (CH_2N) protons and o-aryl hydrogens that arise in a planar conformation.

At the same time the pyridyl function remains co-planar with the double bond to constitute a system of extended conjugation similar to that of the 2-vinylpyridine model. Similarly with the Z-isomer, only the Ar ring and the C-C double bond are co-planar in the preferred conformation and thus the UV spectrum of this isomer strongly resembles that of styrene.

Having determined the configuration of the two isomers by the UV method an explanation of the NMR pattern for each isomer followed. In order to do this, knowledge of the deshielding effects of the 2-pyridyl and phenyl aromatic functions was required. The order of these effects was obtained by observation of the respective chemical shifts of the vinylic protons of the same two models, 2-vinylpyridine (32) and styrene (33) and by comparison to ethylene.



a. 2-vinylpyridine (32) b. styrene (33) c. ethylene

Fig 5.1 Chemical shifts (in ppm) of the vinylic protons of 2-vinylpyridine,styrene and ethylene. (Ison and Casy, 1971)

It is clear from these data that the 2-pyridyl group has a deshielding effect on both vinylic protons but that the phenyl group has a deshielding effect for the vinylic proton in the cis position and a small shielding effect on the trans proton. Both of these screening effects will be reduced the more the ring is turned out of the plane of the double bond.

It may be anticipated from considerations of shielding, that the methylene amino protons cis to 2-pyridyl will have a lower chemical shift that those cis to phenyl or substituted phenyl. This is found to be the case, in the absence of solvating solvents (see later).

In the case of triprolidine (23), Ison and Casy (1971), obtained the following 60 MHz NMR data (Table 5.1)

Table 5.1 60 MHz Chemical shifts, in ppm, for triprolidine base and its oxalate salt

		Vinylic H (t)	CH_2N (d)
Base in $CDCl_3$	E	6.92	3.21
	Z	6.27	3.24
Salt in D_2O	E	6.65	4.02
(oxalate)	Z	6.45	3.95

The vinylic proton of the E-isomer in both the base and the salt was at a lower field, the difference being $\Delta\delta$ 0.65 for the base and $\Delta\delta$ 0.2 for the oxalate salt. No significant difference was seen for the CH_2N signal.

A similar pattern was seen for the hydrochloride salt of triprolidine, run at 270 MHz, in the two solvents, $CDCl_3$ and D_2O (Table 5.2).

Table 5.2 Chemical shifts, in ppm, of triprolidine hydrochloride run in $CDCl_3$ and D_2O (270 MHz)

		Vinylic H (t)	CH_2N (d).
Hydrochloride in $CDCl_3$ [1]			
	E	7.01[2]	3.80
	Z	6.39	3.98
Hydrochloride in D_2O [3]			
	E	6.46	3.86
	Z	6.24	3.78

Footnotes

1 referenced to TMS
2 overlapped aromatic signals
3 referenced to HDO at 4.8 ppm

Again the chemical shift of the vinylic proton of the E-isomer is greater (lower field) than that of the Z-isomer, as expected due to the greater deshielding effect of the 2-pyridyl function on its adjacent group in the trans compound (Fig 5.1a). In this case the difference seen for the salt in D_2O ($\Delta\delta$ 0.22) was smaller than that found for the salt in $CDCl_3$ ($\Delta\delta$ 0.62) - the same pattern as seen for the base ($CDCl_3$) and salt (D_2O) in the 60 MHz data (Table 5.1).

In both examples shown, the largest difference in vinylic chemical shifts is seen using $CDCl_3$ as the solvent. This is probably due to solvation effects of the D_2O on the nitrogen lone pair of the pyridyl ring. Thus when the pyridyl nitrogen of the E-isomer is solvated the non bonded interactions between the nitrogen lone pair and the vinylic proton are raised (Fig 5.2) - these may be avoided by

a reduction in the planarity of the 2-vinylic pyridyl chromophore and as a result deshielding of the vinylic proton is reduced as compared with that seen in the non-solvating solvent, $CDCl_3$. Hence the vinylic proton signal moves upfield from 7.01 ppm (in $CDCl_3$) to 6.46 ppm (in D_2O). Solvation of the pyridyl group in the Z-isomer has very little effect on the vinylic proton.

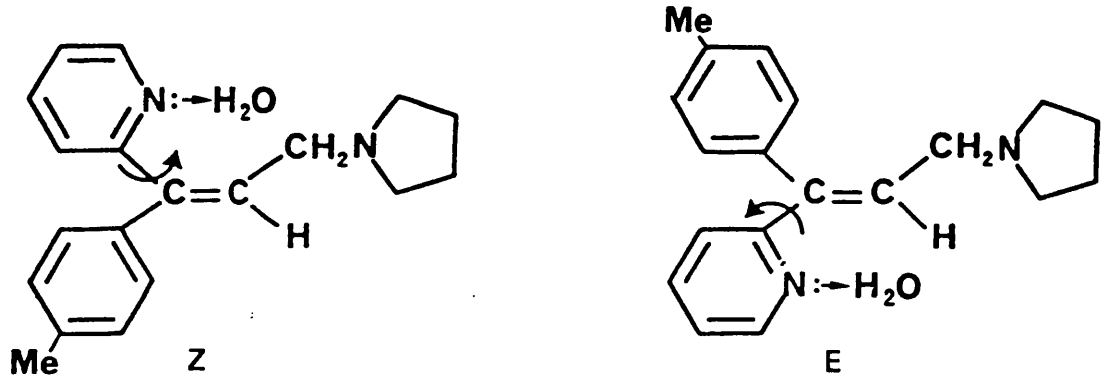


Fig 5.2 Effects of solvation on triprolidine and its Z isomer

The Z-CH_2N protons of the hydrochloride were lower field than those of the E-isomer, in $CDCl_3$, as anticipated, but took the high field position when D_2O was the solvent. Here too, solvating the pyridyl nitrogen will enhance non-bonded interactions between the pyridyl lone pair and CH_2N protons and thus reduce the deshielding influence of the pyridyl ring.

^{1}H-NMR was also used as a method for purity assessment for the samples of triprolidine and its Z-isomer (obtained from B-W). By ^{1}H-NMR, the sample of the Z-isomer of triprolidine was not 100% pure i.e. it showed duplicated signals of the E-isomer, visible at the low field edge of the vinylic triplet and methylene amino doublet (Fig 5.3).

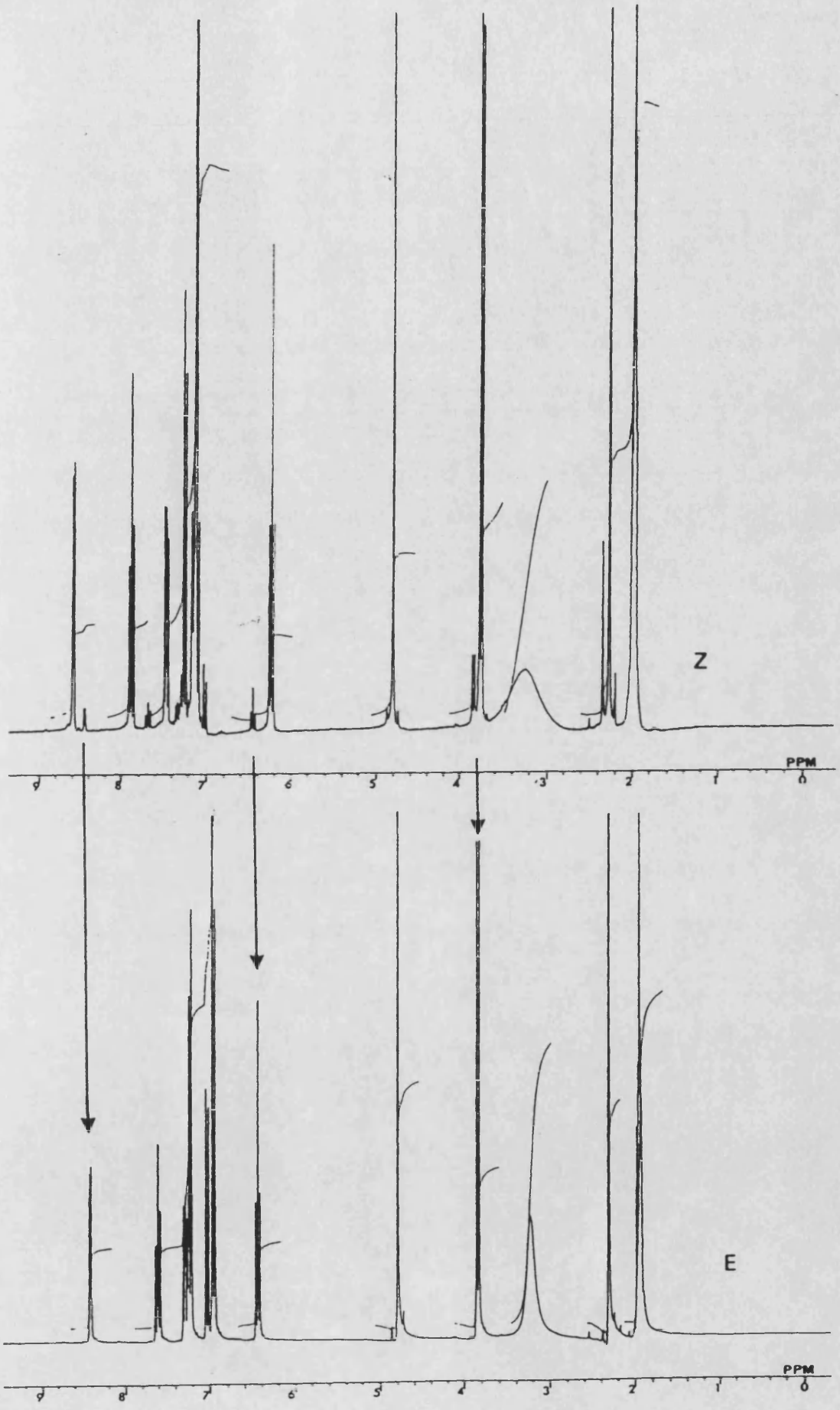


Fig 5.3 ^{1}H-NMR spectrum of E-triprolidine and its Z-isomer

In view of the relative insensitivities of ^{1}H-NMR, a second method of measuring the stereochemical purity of the samples was developed. This involved the use of HPLC, since this had been successfully applied to chiral compounds. The separation of geometric isomers should be less difficult than that of optical antipodes because of their different chemical and physical properties. The HPLC column chosen initially was a Hypersil 5 ODS (25 cm), - but results with this gave very broad tailing peaks, even after the incorporation of ammonium acetate buffer (pH6), an ion pair reagent and KCl into the mobile phase of 25% THF. The tailing found using this column was due to analytes binding the uncapped silanol groups on the silica support.

A more polar column, with a shorter, (C_3) chain i.e. Hypersil 5 CPS (10 cm) was chosen. In this cyanopropyl (CPS) column there are only propyl (C_3) chains attached to the Si support. Therefore more silanol groups are capped and so less tailing is seen because of the reduced likelihood of the analyte finding any free polar groups. Using a mobile phase of 20% THF and 80% water containing KCl (50 mM), Hexanesulphonic acid (10 mM) and phosphoric acid (0.01%), good separation of the isomeric peaks was seen. The choice of wavelength for detection varied with each pair of isomers tested. The actual value used was the isobestic point of the two isomers. In choosing the isobestic point as the detection wavelength, one is assured that the absorption of each isomer is the same and thus the true proportion of each isomer, in a given solution, will be identified. In the case of triprolidine the detection wavelength was 245 nm.

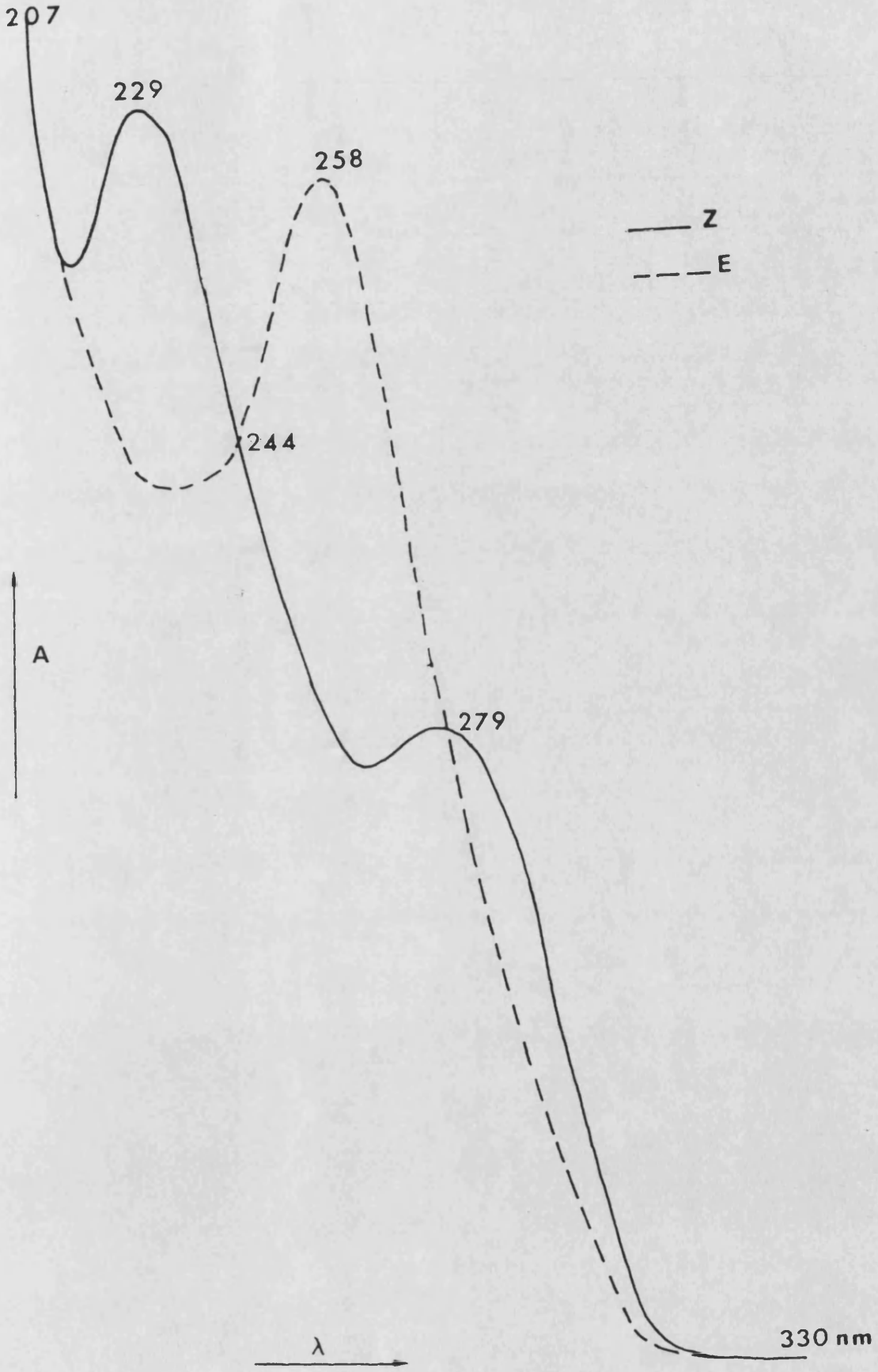


Fig 5.4 UV spectra of the E and Z isomers of triprolidine showing the two isobestic points

By HPLC methods, the original sample of the Z-isomer of triprolidine was shown to have a 5% impurity of the E-isomer (Fig 5.5).

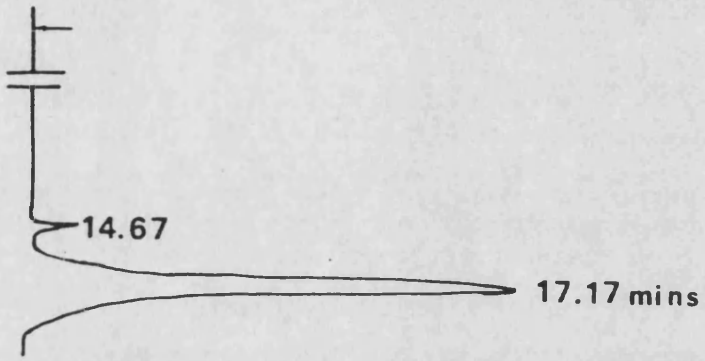


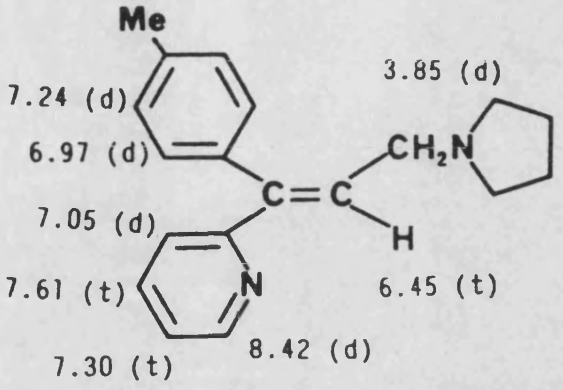
Fig 5.5 HPLC trace of the Z-isomer of triprolidine showing the 5% impurity of the E-isomer

When the material, recrystallized once from absolute ethanol was chromatographed only one peak at 17.5 minutes was recorded. Analysis of the E-triprolidine sample, provided by B-W, showed one peak with a retention time of 13.28 mins.

Another method of establishing the configuration of the isomers was the use of NOE (Nuclear Overhauser Effect) spectroscopy. If a proton is irradiated at a certain frequency it may result in an increase or decrease in the intensity of another proton. The change in intensity only occurs when the irradiated nucleus and that undergoing the intensity change are close in space. This effect is known as the Nuclear Overhauser Effect (NOE) and is important since it gives information about molecular geometry. The NOE is a 'through space effect and may occur irrespective of whether the two nuclei are spin-spin coupled.

The spectra quoted in this thesis are NOE Difference spectra (NOED). Using this method a portion of the spectrum, far removed from the signals of interest, is irradiated and this effect is subtracted from the results obtained on irradiation of the signals of interest. By studying the difference spectra one is assured that any NOE seen is due to the protons being in close proximity to one another and not just an artefact of the technique.

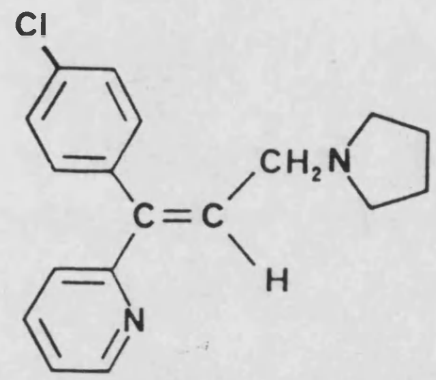
In the case of triprolidine, the following results (Fig 5.6) were obtained confirming well, the configuration as E.



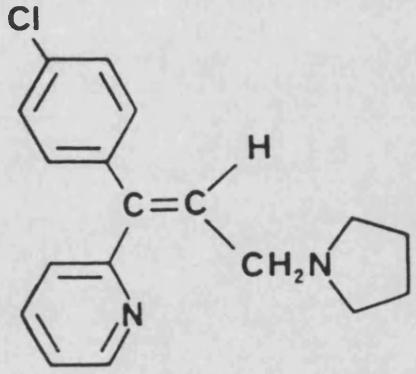
Position of irradiation (ppm)	Resonance assignment	Result
3.9	CH_2N	NOE at 6.97 ppm (A_2 of A_2B_2 signal) and at 6.45 (vinylic CH)
6.45	vinylic CH	NOE at 7.05 ppm signal between the two halves of the A_2B_2 signal (H-3' Py)
6.97	A_2 of A_2B_2 p-tolyl	NOE at 3.9 ppm (CH_2N) and at B_2 (7.24 ppm)
7.24	B_2 of A_2B_2 p-tolyl	NOE at A_2 of A_2B_2 and at 7.61 ppm (H-4' Py)

Fig 5.6 400MHz NOE spectrum for triprolidine (23)

Isomeric samples obtained from R.R. Ison (Thesis, 1970), of the p-chloro analogues of triprolidine showed the same spectroscopic pattern, as previously described.



(24)



(34)

UV analysis of these two isomers (salts in ethanol) showed the E-isomer (24) to have two maxima at 230 (E 14,114) and 274 nm (E 6,686) and the Z-isomer (34), one maximum at 259 nm (E 12,907). This was in agreement with the triprolidine results, the E-isomer showing a comparable result to that of 2-vinyl pyridine.

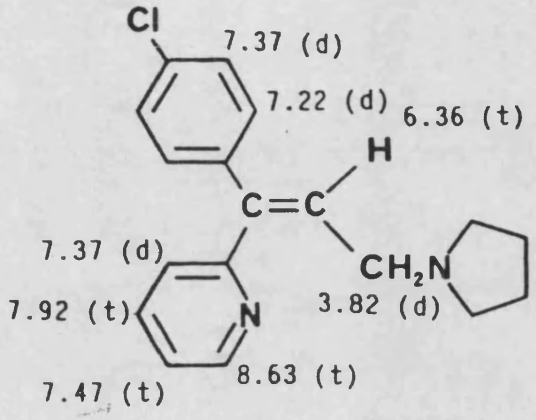
HPLC analysis of these two isomers was carried out at a detection wavelength of 245 nm (the isobestic point). This showed results similar to those obtained with triprolidine. The Z-isomer was retained longer than its E-analogue. The Z-isomer was shown to have a 1.5% impurity of the E-isomer and the E-isomer a 4% Z-impurity, but these were both removed by a single recrystallization from ethanol.

^{1}H-NMR analysis (270 MHz) of the two isomers, (as oxalates in D_2O) showed the following results,

Table 5.3 ^{1}H-NMR chemical shifts of the p-chloro analogue of triprolidine and its isomer as oxalates in D_2O (270 MHz)

	Vinylic H (t)	CH_2N (d)
E-(24)	6.63	3.91
Z-(34)	6.38	3.86

These NMR results also correlate well with those obtained for triprolidine (Table 5.2). Similarly, results from the NOED spectrum confirmed the configuration of one of the two samples - the E-isomer (Fig 5.7), since irradiation of the high field aromatic doublet resulted in a clear NOE at the vinylic proton site and vice versa.



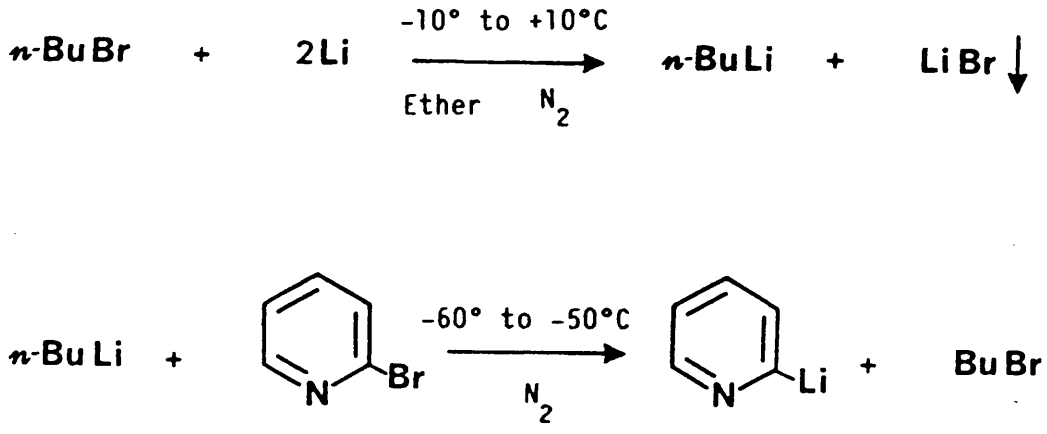
Position of irradiation (ppm)	Resonance assignment	Result
3.82	CH_2N	NOE at the vinylic CH (6.36 ppm)
6.36	vinylic CH	NOE at Ar A_2B_2 (7.22 ppm) and CH_2N (3.82 ppm)
7.2	Ar - A_2B_2	Large NOE at 7.37 ppm and NOE at vinylic CH (6.36 ppm)
7.47	H-5' (pyridyl)	NOE at H-6' (8.63 ppm) and H-4' (7.92 ppm)

Fig 5.7 NOED spectrum for Z p-chloro analogue (34) of triprolidine

Further confirmation of the ability of these techniques to provide suitable methods for configurational assignments of 2-pyridyl amino propene isomers was provided by examination of the UV and ^{1}H-NMR spectra of some other analogues of triprolidine

These compounds were initially prepared using the method of Ison (1970) and Adamson and Billinghurst (1950). 2-Pyridyl-lithium was the reagent used for the introduction of the 2-pyridyl group into the alcohol structure (35) (Scheme 2). The synthesis of 2-pyridyl lithium was by one of two methods.

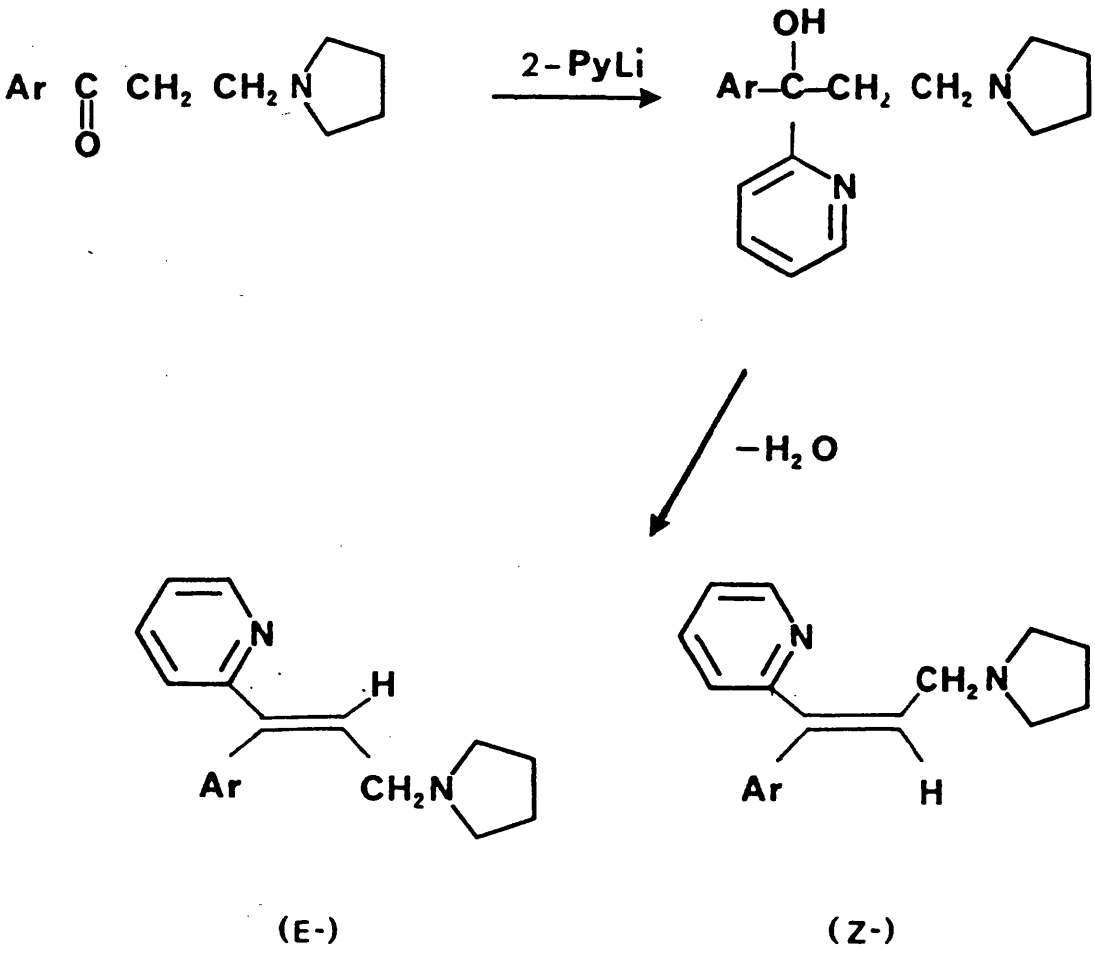
The first, involved the preparation of an ethereal solution of n-butyl lithium (BuLi) followed by its treatment with 2-bromopyridine under nitrogen at -60°C - -50°C.



Scheme 1

The second method of synthesis of 2-pyridyl lithium, was by the direct addition of commercial BuLi (2.5 M in hexane) to 2-bromopyridine in dry ether (Pathway 2. Scheme 1). Both methods were successful - but the latter was eventually taken as the method of choice.

The 2-pyridyl lithium was reacted with the appropriate Mannich ketone (obtained from an aryl substituted acetophenone, pyrrolidine HCl and paraformaldehyde) to produce the respective alcohol.



(E-) (Z-)

Scheme 2

Dehydration of the alcohol was effected by heating it in a solution of H_2SO_4 (85%) at 120°C for varying lengths of time. The basic reaction product was isolated, after clean up through an acid/base cycle, and in most cases, negligible amounts of starting t-alcohol were found. Acidification of the oily basic reaction mixture with a saturated solution of oxalic acid in acetone followed by fractional recrystallization yielded pure oxalate salts of each isomer, in each case.

Configurational assignments of the two oxalate isomers were made, as previously described from observation of their UV and ^{1}H-NMR spectra.

The un-substituted phenyl analogue (36) of triprolidine was synthesized as previously described. Varying the dehydration time in 85% H_2SO_4 at 120°C did not give one pure isomer - but in all cases gave mixtures of varying proportions. Using HPLC, (at 245 nm) to quantify the isomeric ratio, is was found that 10 mins dehydration gave 60%, 1 hour gave 68% and 3 hours gave 80% of the Z-isomer (37) and negligible amounts of starting alcohol were found. After numerous recrystallizations of the oxalate salts - the highest proportion of Z-isomer in the product was 70%. After 4 hours dehydration and five recrystallizations from ethanol a pure sample of E-isomer (36) (99% by HPLC) was obtained. UV analysis showed two maxima at 276 and 226 nm. The ^{1}H-NMR details are shown in Table 5.4

Table 5.4 ^{1}H-NMR chemical shifts for analogues of triprolidine

Analogue		Vinylic CH (t)	CH_2H (d)
Phenyl*			
E- (36)	base	6.98	3.26
	oxalate	6.69	4.00
Z- (37)	base	6.35	3.20
	oxalate	6.38	3.84
p-Bromophenyl*			
E- (38)	oxalate	6.69	4.00
Z- (39)	oxalate	6.35	3.84
p-Ethylphenyl*			
E- (40)	base	6.94	3.22
	oxalate	6.63	3.99
Z- (41)	base	6.33	3.26
	oxalate	6.33	3.83

* Aryl group which replaces p-tolyl of triprolidine

Base samples were run in $CHCl_3$, oxalate salts in D_2O

Synthesis of the p-bromo derivative (38) of triprolidine followed the same pathway as described, although formation of the alcohol was carried out in an ether/THF mixture, because the pBr-Mannich ketone was not soluble in ether. Varying dehydration conditions were evaluated. After four hours at 120°C (85% H_2SO_4) and three recrystallizations of oxalate salts from absolute ethanol pure E-isomer (38) was collected. This was identified by its UV spectrum, showing maxima at 230 and 275 nm and it's ^{1}H-NMR parameters(Table 5.4).

Isolation of pure Z-isomer (39) was more difficult. Dehydration times of 30 and 60 minutes raised the percentage of Z-isomer but after three recrystallizations of the oxalate salt, from ethanol, this did not alter greatly. The ^{1}H-NMR details given in Table 5.4 are for the Z-isomer (70%) plus E impurity.

HPLC analysis was carried out at 245 nm - the isobestic point for the three previous examples. This confirmed the mixture proportions that were calculated using ^{1}H-NMR. Again it was the Z-isomer that was retained the longest on the column.

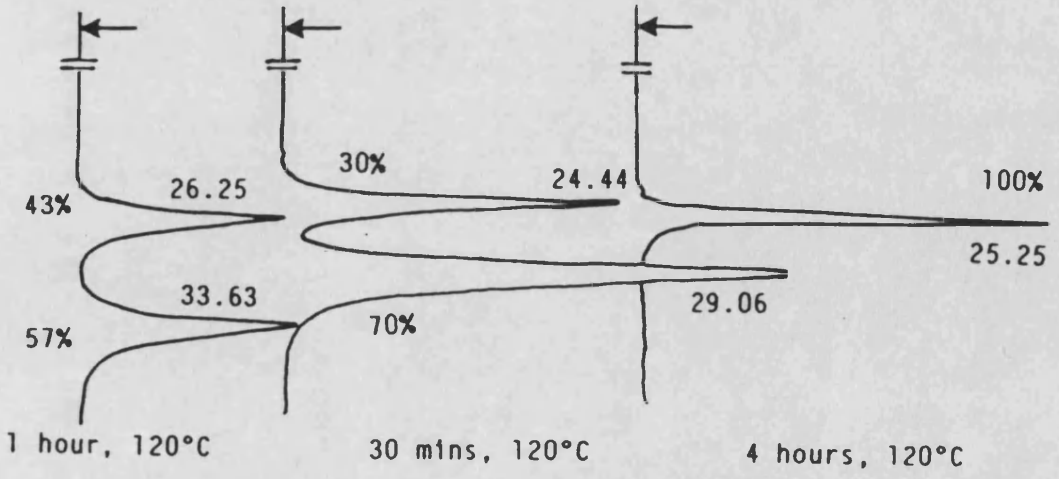


Fig 5.8 HPLC trace for the p-bromo derivative (38) of triprolidine after varying dehydration times.

The p-ethyl derivative (40) was synthesized as previously described via the Mannich ketone and its conversion to the tertiary alcohol. Dehydration of the alcohol with 85% H_2SO_4 at 120° followed, and again varying proportions of the two isomers were obtained, depending on the length of time the alcohol was subjected to dehydration. In this p-ethyl analogue 1 hour at 120°C was sufficient to produce pure E-isomer (40). Identification of this isomer was as before. UV results indicated two maxima at 229 and 275 nm, the ^{1}H-NMR resonances are shown in Table 5.4, and HPLC analysis at 245 nm showed one peak at approximately 20 mins retention.

Dehydration of the alcohol for a 10 minute period gave a first crop consisting of a mixture of isomers 60 (20 mins R_T): 40 (30 mins R_T) (E:Z) by HPLC. Recrystallization from ethanol increased the proportion of E-isomer and after three recrystallizations more pure E-isomer was collected. The second and third crops from the 10 minute dehydration showed an increase in Z-isomer (41) 92% (30 mins by HPLC). Similarly collection of the solid from the mother liquors of the recrystallizations yielded a sample also rich in the Z-isomer (41) (93% by HPLC). The ^{1}H-NMR parameters are shown in Table 5.4. The UV spectrum showed one maximum at 260 nm.

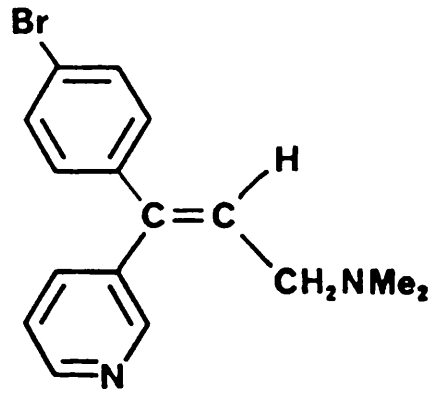
All the results from these 2-pyridyl analogues confirm the results for the configurational analysis of triprolidine. The vinylic and CH_2N protons resonances of the oxalate salts in D_2O for the E-isomer are always lower field than their Z- counterpart.The UV parameters also followed the same pattern as observed for triprolidine, the E-isomer showing two maxima comparable to the spectrum of 2-vinyl pyridine and the Z-isomer one maximum as for styrene.

HPLC analysis has shown consistent results throughout - the Z-isomer being retained longer than the E in all the examples given.

5.3 3-Pyridyl compounds

5.3.1 Introduction

A 3-pyridyl analogue of triprolidine is commercially available, namely Zimeldine (27), a compound which has the Z-configuration (Abrahamson et al, 1976).



(27)

Zimeldine is marketed for use as an antidepressant agent although it does have a weak antihistaminic action (Hall and Ogren, 1984)

Table 5.5 Pharmacological data for zimeldine and some analogues (Hall and Ogren, 1984)

	Histamine antagonist effects (IC_{50} μm)	
	GP ileum	[^{3}H]-mepyramine binding cerebral cortex
Zimeldine (27)	20.4	2.9
E-isomer (42)	1.8	0.4
NHMe of Z-(27)	42.3	8.3
NHMe of E (27)	21.2	3.4

The data of Table 5.5 shows that the corresponding E-isomer (42) is the more active as an H_1-antagonist, as was the case for the 2-pyridyl compounds. The E-isomer is 11 times more potent than zimeldine at guinea-pig ileum sites and 7 times the more effective at displacing [^{3}H]-mepyramine from rat cerebral cortex. The overall potency, however, of the more active E-isomer was only 6% that of brompheniramine. Similar results were obtained for the corresponding methylamino analogues.

5.3.2 Configurational analysis

Abrahamson et al (1976) have reported the X-ray analysis of zimeldine, identifying it as the Z-isomer. Our analyses have involved the use of UV, ^{1}H-NMR, NOED spectra and HPLC (as employed for the 2-pyridyl compounds) to confirm configuration in this series.

In order to identify the configuration of the hydrochloride salts of zimeldine and its E-isomer (kindly supplied by Astra Pharmaceuticals, Sweden), it was necessary to study the UV and ^{1}H-NMR spectra of model compounds. The models chosen were 3-vinylpyridine (43) and styrene (33) (as used previously). The UV spectrum of 3-vinylpyridine (Organic Electronic Spectral Data.) showed two maxima (as did 2-vinylpyridine) at 238 nm (ε 12,600) and 278 nm (ε 2,500). Styrene showed only one maximum at 254 nm.

The UV spectrum of zimeldine and its E-isomer showed a similar pattern (Fig 5.9). Zimeldine (27) showed a single maximum at 260 nm (ε 12,062), similar to that of styrene, and the E-isomer (38) showed a maximum at 232 nm (ε 14,119), similar to that of 3-vinylpyridine. The second, longer wavelength maximum of 3-vinylpyridine was not visible. These results indicate that a styrene-like chromophore operates in zimeldine, and one akin to 3-vinylpyridine in the related E-isomer, and support the configurational assignments by the same arguments as those applied to the 2-pyridyl series (5.2).

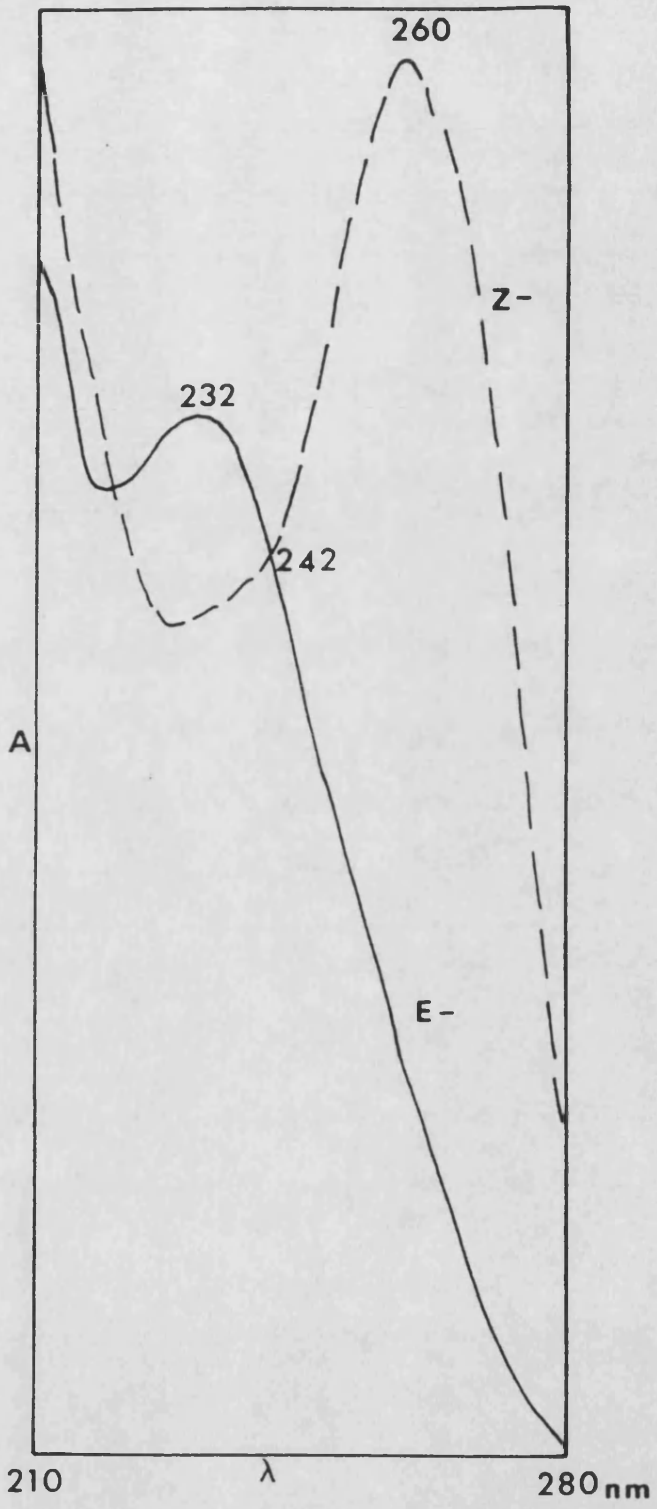
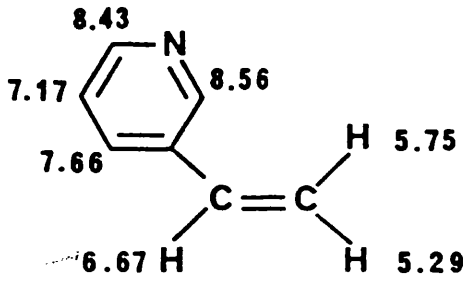


Fig 5.9 UV spectrum of zimeldine HCl (27) and its E-isomer (42) (in H_2O)

^{1}H-NMR analysis of the two isomers was aided by the reported 60 MHz ^{1}H-NMR analysis of the model compound 3-vinylpryidine (43) (Klemm and McCoy, 1969). This report identified the same deshielding pattern for this 3-pyridyl compound as was seen for its 2-pyridyl analogue i.e. cis-H more deshielded than the trans-H ($\Delta\delta$ = 0.46 ppm at 60 MHz).



(43)

Hence the same arguments, as previously reported, will still apply.

Unfortunately in the case of zimeldine and its E-isomer, the differences between the isomeric vinylic and methylamino proton resonances were only small and therefore of less value for the configurational argument. Table 5.6 shows, however, that the signals were resolved and thus ^{1}H-NMR was useful for isomeric purity assessment.

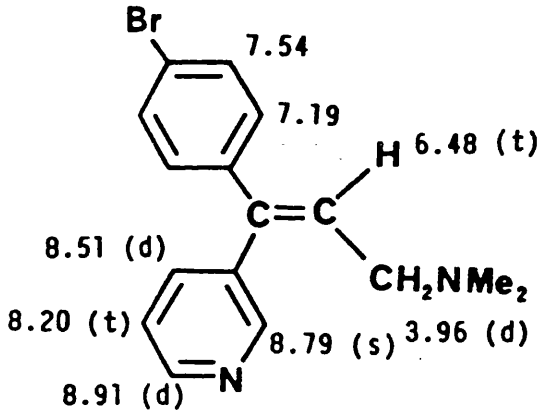
Table 5.6 ^{1}H-NMR chemical shifts of zimeldine HCl and some oxalate analogues, in D_2O. (270 MHz)

		Chemical Shifts (ppm from TMS)	
		vinylic CH	CH_2N
zimeldine	E (42)	6.47	3.89
	Z (27)	6.51	3.90
phenyl analogue	E (45)	6.43	3.97
	Z (44)*	6.38	3.90
p-methyl analogue	E (46)	6.38	3.98
	Z *	(6.35)	3.89

Footnotes:

* Results obtained from an isomeric mixture of E and Z.

The use of NOED spectroscopy again proved useful in confirming the configuration of the 3-pyridyl isomers and Fig 5.10 shows the results obtained for zimeldine.2HCl in D_2O. By irradiating the vinylic proton - a clear NOE was seen at the p-bromophenyl protons and vice versa - indicating the presence of Z-isomer.



Position of irradiation (ppm)	Resonance assignment	Result
3.96	CH_2N	No significant NOE except NMe_2 signal
6.48	vinylic CH	NOE at A_2 (7.19 ppm)
7.18	A_2 of A_2B_2	Excellent NOE for vinylic CH (6.48 ppm) and B_2 (7.53 ppm)
7.53	B_2 of A_2B_2	Clear NOE for A_2 (7.18 ppm)

Fig 5.10 NOED spectrum for zimeldine dihydrochloride (27)

HPLC, using the same chromatographic conditions as for 2-pyridyl compounds, again proved useful in the measurement of isomeric purity. Using the isobestic point, 242 nm, as the detection wavelength, both zimeldine hydrochloride and its E-isomer gave chromatograms that consisted of one peak only and showed no evidence of the other isomer (Fig 5.11).

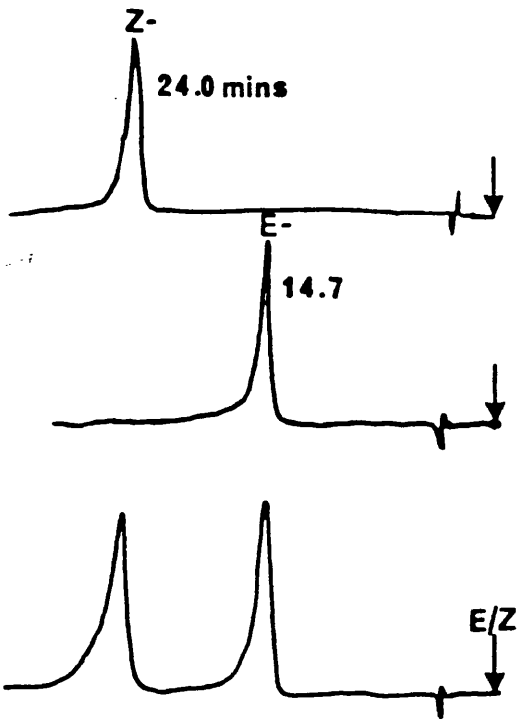


Fig 5.11 HPLC traces of zimeldine hydrochloride and its E-isomer

Once again, the Z-isomer was retained the longest on the column, as in the 2-pyridyl compounds. This could be a useful indication of the configuration of a novel compound.

The synthesis of some analogues of zimeldine was undertaken. Reports (Astra Patent, 1978) of the synthesis of some analogues of zimeldine describe a pathway similar to that previously employed for triprolidine, and thus this was the chosen route. This pathway included the formation of a Mannich base followed by reaction of this Mannich ketone with 3-pyridyl lithium. Preparation of 3-pyridyl-lithium (Gilman and Spatz, 1951) involved the dropwise addition of 3-bromo-pyridine, in anhydrous ether, to a rapidly stirred solution of butyl lithium (BuLi) in ether at -5-0°C. Each addition of 3-bromo-pyridine formed a yellow flocculant precipitate which gradually changed to red/brown. Dropwise addition of the Mannich ketone base in anhydrous ether, to 3-pyridyl-lithium, at -50°C, followed by acid/base extraction of the reaction mixture yielded the t-alcohol base as a yellow oil.

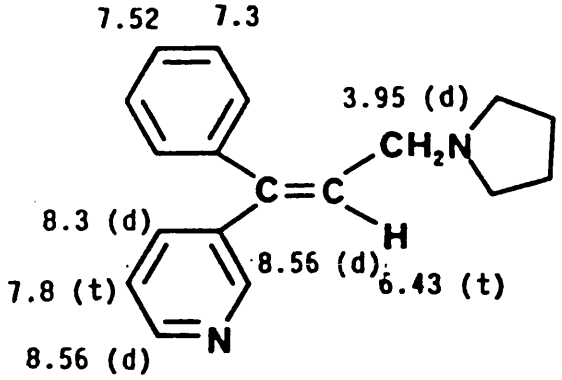
The t-alcohol was dehydrated in the same way as the 2-pyridyl analogues, using 85% H_2SO_4 at 120°C for varying lengths of time. Acidification of the basic reaction mixture with a saturated solution of oxalic acid, in acetone, yielded an isomeric mixture of the oxalate salts.

Analysis of the isomeric purity of these oxalate salts was carried out by ^{1}H-NMR and HPLC using a detection wavelength of 242 nm (the isobestic point for zimeldine and its E-isomer).

The unsubstituted phenyl analogue of zimeldine (44) (Fig 5.12) was synthesized as previously described . Dehydration of the t-alcohol overnight at room temperature, with 85% H_2SO_4 yielded only the starting alcohol. A 30 minute dehydration at 120°C, however, showed an 80% majority of one isomer. After two recrystallizations of the oxalate salt, from absolute ethanol, a sample 90% rich in one isomer was obtained. Similarly, using a dehydration time of 1 hour, at 120°C, followed by three recrystallizations from EtOH, a sample 91% rich, by ^{1}H-NMR and HPLC, in the same isomer was obtained.

Both the E and the Z, vinylic and methylene amino proton resonances were very close (Table 5.6). It was clear that the lower field vinylic signal was the more intense. Identification of the configuration of the isomer in the highest concentration (91%) was difficult using ^{1}H-NMR because of the small $\Delta\delta$ value of the vinylic resonances. Zimeldine (the Z isomer) shows a lower field vinylic signal than its E-isomer (Table 5.6).

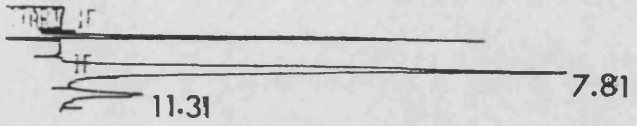
UV analysis, in ethanol showed two peaks at 226 nm (ε 12,617) and 244 nm (ε 10,721). This spectrum resembled that of the E-analogue of zimeldine (and 3-vinyl-pyridine) and would therefore suggest that the product was the E-isomer (45). This contradicts the initial conclusions made from the ^{1}H-NMR data which points to a Z-configuration (44). Obviously more data was necessary.
The use of NOED spectroscopy again proved very helpful in this configurational analysis. The NOED data showed the sample to be the E-isomer (45) (Fig 5.12) since irradiation at the vinylic CH caused an NOE of the pyridyl H-2 and H-4 protons.



Position of irradiation (ppm)	Resonance assignment	Result
3.95	CH_2N	NOE at vinylic CH (6.43 ppm)
6.43	vinylic CH	Clear NOE at H-4' (8.3 ppm) and H-2' (8.56 ppm) - pyridyl
7.52	aromatic	NOE at phenyl signal (7.2 ppm)
8.56	pyridyl H-2'	NOE at phenyl signal (7.52 ppm) and at vinylic CH (6.43 ppm)
7.3	phenyl	NOE at CH_2N (3.95 ppm and some pyridyl signals
8.3	pyridyl H-4'	NOE at vinylic CH (6.43 ppm and at pyridyl H-5'

Fig 5.12 NOED spectrum for E-phenyl (N-pyrrolidino) analogue of zimeldine (44)

Confirmation of the presence of the E-isomer (45) was gained from HPLC analysis of the oxalate sample. The largest peak of the chromatogram (91% by area) was that which was retained for the shortest time (7.81 mins) (Fig 5.13). In all previous examples the E-isomer has been the least retained by the column.



STOP

AREA%

RT	AREA	TYPE	AR/HT	AREA%
7.81	7826600	PB	0.526	91.38%
11.31	738090	BB	0.673	8.618

Fig 5.13 HPLC trace for the isomers of the phenyl analogue of zimeldine (44 and 45)

In the synthesis of the p-tolyl analogue of zimeldine, dehydration of the alcohol for 15 minutes at 120°C had no effect and only starting alcohol remained. A 30 minute dehydration time, at 120°C, was, however, sufficient to produce a 90:10 ratio of isomers. Three recrystallizations from absolute ethanol yielded a maximum of 96% of the major isomer.

UV analysis of this compound showed only one peak at 227 nm (ε 14,413) suggesting the E-isomer was dominant. ^{1}H-NMR showed only one vinylic triplet centred at 6.37 ppm (in earlier, less pure samples another overlapping triplet at higher field was noticeable) but showed two methylene amino doublets, the largest being at lower field. Comparing this to the ^{1}H-NMR results for the unsubstituted analogue this would confirm the UV results i.e. the presence of the E-isomer (46). HPLC analysis, again showed the major peak to be that which eluted first - indicating presence of the E-isomer (46).

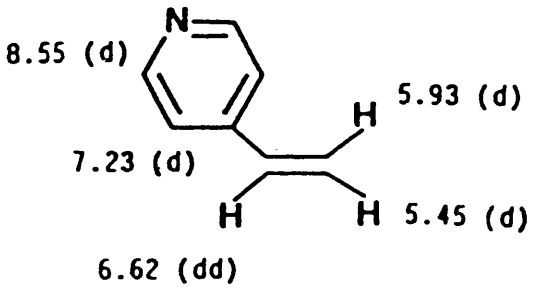
5.4 4-Pyridyl compounds

Further modifications to the triprolidine structure are now discussed. These involve the incorporation of a 4-substituted pyridyl ring, as a replacement of the 2-pyridyl group, into the propene structure.

Synthesis of these compounds was as described in Scheme 2 (Page 191) but using 4-substituted pyridyl lithium. Preparation of the 4-pyridyl lithium required the initial liberation of 4-bromopyridine from its hydrochloride salt by basification with K_2CO_3 followed by ethereal extraction. The 4-bromopyridine was obtained by removing the ether *in vacuo* just before use. 4-Bromopyridine, in anhydrous ether, was added dropwise to a solution of n BuLi, in ether, at -50- -60°C to produce a yellow suspension. This was left stirring for 1 hour, after which time the usual Mannich ketone base in dry ether was added dropwise. The reaction was left to rise to room temperature and then left stirring overnight. The 4-pyridyl tertiary alcohol product was dehydrated, as previously described, varying the conditions to alter the proportions of each isomer. Purification of the reaction mixture was by recrystallization of the oxalate salts.

As an aid to the configurational assignment of these oxalate salts, the model compound 4-vinylpyridine (47) was used. UV analysis of this model indicated one large maximum at 242 nm and a shoulder at 263 nm. The difference between the λ max for 4-vinyl-pyridine and that of styrene (the model compound for the other isomer) was only small and therefore UV analysis may be of limited use for configurational analysis of these 4-pyridyl analogues.

^{1}H-NMR analysis of 4-vinylpyridine, in $CDCL_3$, showed a similar pattern as 2- and 3-vinylpyridine.



(47)

Again the vinylic proton cis to the pyridyl ring was lower field that the trans proton ($\Delta\delta$ 0.48 ppm at 270 MHz), because of the greater deshielding effect of the pyridyl function on this adjacent proton.

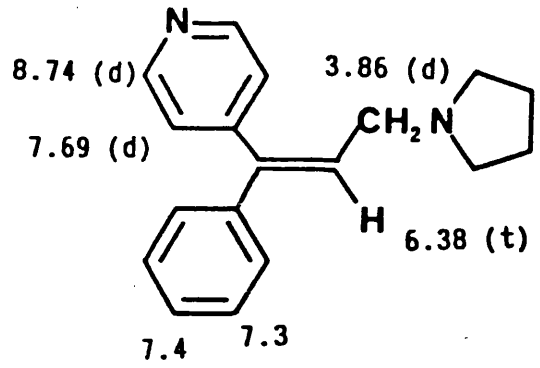
The tertiary alcohol of the unsubstituted phenyl analogue (48) was dehydrated with 85% H_2SO_4, for 30 minutes and 1 hour, at 120°C. Under these conditions the reaction mixture was found to contain 80% of one isomer. Three recrystallizations from absolute ethanol yielded samples that by ^{1}H-NMR were pure. The ^{1}H-NMR results for the vinylic and methyleneamino signals are detailed in Table 5.7. By comparison of the ^{1}H-NMR data obtained from the model compound, study of the vinylic proton chemical shifts, of the synthesized compound, would indicate the presence of the Z-isomer (48) (Fig 5.14) since the higher field triplet was greatest intensity (in the earlier less pure samples).

Table 5.7 Chemical shifts, in ppm, of the oxalate salts of (48) and (49), run in D_2O at 400 MHz (Dehydration times for the t-alcohol are shown in parentheses)

Compound	Chemical Shifts (ppm from TMS) vinylic proton (t)	CH_2N (d)
Z- (48) (30 mins, 1 hour)	6.41	3.90
E- (49) (4 hour)	6.77	4.00

UV analysis of these recrystallized samples show only one maximum at 242 nm (ε 8,050). Since the λmax for both model compounds, 4-vinyl-pyridine and styrene, are very similar, it is not possible to assign the configuration for this compound using UV data.

Evidence of configuration was obtained most directly by the aid of NOED spectroscopy. Using this technique, the sample was identified as being the Z-isomer (48), since irradiation of the vinylic signal caused an NOE at the position of the phenyl signals and irradiation of the CH_2N protons position resulted in an NOE at the H-3' and H-5' pyridyl protons (Fig 5.14).



Position of irradiation (ppm)	Resonance assignment	Result
3.86	CH_2N	NOE for vinylic proton (6.38 ppm)
6.38	vinylic CH	NOE high field phenyl signal (7.3 ppm) and CH_2N signal (3.86 ppm)
7.4	phenyl CH	NOE for high field phenyl signals (7.3 ppm)
7.3	phenyl CH	Clear NOE at vinylic CH (6.38 ppm) and low field signals
7.69	H-2' and H-6' pyridyl	Large NOE for other Py protons (8.74 ppm). Small NOE for CH_2N (3.86 ppm) and high field phenyl aromatics.
8.74	H-3' and H-5' pyridyl	Large NOE for other pyridyl protons (7.69 ppm)

Fig 5.14 NOED spectrum for (48)

Dehydration of the alcohol for the more prolonged period of 4 hours at 120°C gave a sample 90% rich in a different isomer, the E isomer (49). The ^{1}H-NMR spectrum of this product showed lower field chemical shift values for the vinylic and methylene amino protons, compared with those of the isomer isolated under less vigorous dehydration conditions.

Attempts to dehydrate the p-tolyl 4-pyridyl analogue were unsuccessful. Dehydration of the t-alcohol for a 4 hour period resulted in an intangible gummy residue which could not be purified.

5.5 Experimental Details

5.5.1 Introduction

^{1}H-NMR spectra were recorded on a JOEL GX 270 MHz Fourier Transform (FT) NMR spectometer unless otherwise stated. The following abbreviations are used to describe the appearance of the signals in the ^{1}H-NMR spectra: singlet, s; doublet, d: triplet, t; quartet, q; multiplet, m.

^{13}C-NMR spectra were recorded on a JOEL GX 270 FT NMR spectrometer operating at 67.8 MHz unless otherwise stated. The multiplicity of the resonances was obtained from DEPT (Distortionless Enhancement by Polarization Transfer) and INEPT (Insensitive Nuclei Enhanced by Polarization Transfer) spectra in which the phase of the signal indicated the number of protons attached to the carbon atom giving rise to that signal. (See 3.6.3 - dehalogenation of chlorpheniramine)

The Infra-Red spectra (liquids as films, solids as KBr discs or Nujol mulls) were recorded on a Unicam SP1025 spectrometer.

HPLC data quoted are quoted as retention times (in minutes) measured on a 10cm Hypersil (5μm) CPS column, using a mobile phase of 20% THF, 80% water containing 50mM KCl, 10mM Hexane Sulphonic acid and 0.1% H_3PO_4. The detection wavelength being the isobestic point for the isomers being resolved.

Mass spectra were measured on a VG micromass 7070 E mass spectrometer operating at 70 EV El.

Elemental analyses were carried out by Butterworth Laboratories Ltd, Middlesex.

Melting points were recorded on a Gallenkamp apparatus, and are uncorrected.

5.6 Synthesis of the 'Triprolidine' analogues

5.6.1 Synthesis of starting Mannich ketone

5.6.1.1 Preparation of 3-(1-pyrrolidino)-1-p-tolylpropan-1-one (50)

An aqueous solution of pyrrolidine hydrochloride was prepared by careful acidification of pyrrolidine free base (35.6 g; 0.5 M) with concentrated HCl, cooling in an ice-bath. Paraformaldehyde (22.0 g; 0.7 M), p-methyl-acetophenone (60 g; 0.448 M) and ethanol (100 ml) were then added to the reaction flask and the mixture heated under reflux for 13 hours. Cooling to 0°C and dilution with ether failed to separate a solid product. A Dean-Stark head was attached to the reaction vessel and after addition of Toluene (200 ml) the water present was removed by azeotropic distillation (over 8 hours). On cooling a brown crystalline product formed. Filtration followed by trituration with ethylacetate and recrystallization from ethanol/ether gave the pure 3-(1-pyrrolidino)-1-p-tolylpropan-1-one hydrochloride (75 g; 65%), m.p. 165°C (Ison, 1970 gave mp 167°C). ^{1}H-NMR 7.87 (d) 2H, 7.28 (d) 2H, 3.72 (t) 2H, 3.55 (t) 2H, 3.3 (broad) 4H, 2.4 (s) 3H, 2.2 (s) 4H.

5.6.1.2 Preparation of 3-(1-pyrrolidino)1-phenylpropan-1-one (51)

Acetophenone (30 g; 0.25 M), paraformaldehyde (9.9 g; 0.33 M) and acidified pyrrolidine (17.8 g; 0.25 M) were heated under reflux for 6 hours. On cooling the crude crystalline Mannich ketone hydrochloride separated (45.2 g; 69%), m.p. 94-95°C, ^{1}H-NMR 7.98 (d) 2H, 7.68 (t) 1H, 7.56 (t) 2H, 3.71 (m) 2H, 3.59 (s) 4H, 3.17 (m) 2H, 2.18 (m) 4H.

5.6.1.3 Preparation of 3-(1-pyrrolidino)-1-p-ethylphenylpropan-1-one (52)

p-Ethyl acetophenone (37.2 g; 0.25 M), paraformaldehyde (11.7g; 0.39 M) and acidified pyrrolidine (20 g; 0.28 M) were heated under reflux for 12 hours. Removal of the water by azeotropic distillation over 8 hours, cooling, trituration with ethyl acetate and recrystallization from ethanol yielded the pure Mannich ketone hydrochloride (53.2 g; 79%) m.p. 151°C, ^{1}H-NMR 7.95 (d) 2H, 7.45 (d) 2H, 3.63 (s) 4H, 3.47 (broad) 4H, 2.74 (q) 2H, 2.14 (S) 4H, 1.26 (t) 3H.

5.6.1.4 Preparation of 3-(1-pyrrolidino)-1-p-bromophenylpropan-1-one (53)

p-Bromoacetophenone (37.5 g; 0.19M), paraformaldehyde (8.82 g; 0.3 M), acidified pyrrolidine (15 g; 0.2 M) and ethanol (50 ml) were heated under reflux for 10 hours. The water was then removed by azeotropic distillation under a Dean-Stark head for 6 hours. Trituration of the solid with ethyl acetate and recrystallization from ethanol yielded the Mannich ketone hydrochloride (56 g; 93%) m.p. 190°C ^{1}H-NMR, 7.83 (d) 2H, 7.60 (d) 2H, 3.17 (t) 2H, 2.90 (t) 2H, 2.55 (t) 4H, 1.80 (q) 4H.

of intermediate 2-pyridyl tertiary alcohols

of 1-(2-pyridyl)-1-(p-ethylphenyl)-3-(1-pyrrolidino) (54)

lithium in hexane (4.1g, 0.065M) was added dropwise to ridine (6.91g, 0.047M) under nitrogen at -60°C. The mixture ed at -60°C for 1 hour and an ethereal solution of the 0g, 0.04M) was added, with stirring, over a 30 minute The mixture was stirred at -50°C for 1 hour and then allowed to room temperature (overnight). The reaction mixture was with iced water, acidified with N-HCl (6M) and then ed with ether (3 x 100mls). The aqueous layer was basified OH (5M) and the product extracted with ether. The ether layer hed with water (2 x 25ml), dried ($MgSO_4$) and evaporated reduced pressure to yield a cream solid of 1-(2-pyridyl)-1-lphenyl)-3-(1-pyrrolidino)-propan-1-ol (54) (4g, 28%), m.p. °C, ^{1}H-NMR 8.53 (d, 1H, Py H-6'), 8.00 (t, 1H, Py H-4'), d, 1H, Py H-3'), 7.61 (t, 1H, Py H-5'), 7.50 (d, 2H, A_2 of 7.20 (d, 2H, B_2 of A_2B_2), 2.50 (m, 10H), s, 4H, pyrrolidino), 1.20 (t, 3H, Ar-CH_2-$\underline{CH_3}$).

-pyridyl)-1-(phenyl)-3-(1-pyrrolidino)propan-1-ol (55)

his tertiary alcohol was prepared in the same way as the nditions outlined in Table 5.8. The product separated as a brown l but formation of the oxalate salt yielded a white solid of 2-pyridyl)-3-(phenyl)-3-(1-pyrrolidino)propan-1-ol (55) (4.6g. 38%), p. 164 -165°C, ^{1}H-NMR, 8.53 (d, 1H, Py H-6'), 8.16 (t, 1H, Py 4'), 8.84 (d, 1H, Py H-3'), 7.60 (t, 1H, Py H-5'), 7.4 (m, 5H, henyl) 3.60 (m, 2H), 3.15 (m, 2H), 2.94 (m, 4H), 2.00 (s, 4H, yrrolidino)

Compound No	X	Y	Base Ketone	Bromo-Pyridine	BuLi	Yield
55	H	2-pyridyl	0.05M (10g)	0.06M (8.7g)	0.08M (32mls)	38%
	Me	2-pyridyl	0.046M (10g)	0.05M (7.4g)	0.08M (31.3mls)	37%
54	Et	2-pyridyl	0.04M (10g)	0.05M (6.9g)	0.07M (29.3mls)	28%
56	Br	2-pyridyl	0.06M (15.9g)	0.07M (10.22g)	0.10M (40mls)	20%
57	H	3-pyridyl	0.05M (10g)	0.06M (8.7g)	0.08M (32mls)	52%
58	Me	3-pyridyl	0.046M (10g)	0.055M (8.0g)	0.075M (30mls)	37%
59	H	4-pyridyl	0.05M (10g)	0.063M (9.2g)	0.066M (26.3mls)	24%
60	Me	4-pyridyl	0.046M (10g)	0.06M (9.0g)	0.075M (30mls)	20%

Table 5.8 Experimental details for preparation of propan-1-ols

Footnotes

BuLi = 2.5M solution in hexane

5.6.2.3 1-(2-pyridyl)-1-(p-bromophenyl)-3-(1-pyrrolidino)propan-1-ol (56)

Prepared as for (54), using the conditions outlined in Table 5.8. The final extraction with ether yielded a brown oil which was azeotroped with acetone, redissolved in anhydrous ether and scratched to give a brown solid. Recrystallisation with absolute ethanol yielded a cream solid (3g, 20%), m.p. 140°C, ^{1}H-NMR, 8.44 (d, 1H, Py H-6'), 7.63 (d, 1H, Py H-3'), 7.55 (t, 1H, Py H-4'), 7.45 (d, 2H, A_2 of A_2B_2), 7.32 (d, 2H, B_2 of A_2B_2), 7.00 (t, 1H, Py H-5'), 2.3 - 2.6 (m, 8H, $\underline{CH_2}$-$\underline{CH_2}$-N($\underline{CH_2}$)$_2$), 1.65 (s, 4H, pyrrolidino).

5.6.3 Preparation of 3-pyridyl tertiary alcohol intermediates

The following compounds were prepared in the usual manner (5.6.2.1) but using 3-pyridyl lithium and the appropriate Mannich ketone . Details of proportions and yield are given in Table 5.8.

5.6.3.1 1-(3-pyridyl)-1-(phenyl)-3-(1-pyrrolidino)propan-1-ol (57)

m.p. 141°C, ^{1}H-NMR 8.74 (s, 1H, Py H-2'), 8.50 (very broad s, 1H, disappears on deuteration), 8.41 (d, 1H, Py H-6'), 7.78 (d, 1H, Py H-4'), 7.46 (d, 2H, phenyl), 7.30 (t, 2H, Py H-5' and phenyl), 7.21 (t, 2H, phenyl), 2.62 (t, 2H, CH_2-$\underline{CH_2}$), 2.43 (t, 2H, $\underline{CH_2}$-CH_2), 1.75 (s, 4H, pyrrolidino).

Found C, 77.2%; H, 8.25%; N, 9,59%. Calculated $C_{18}H_{22}N_2O$, C, 76.56%, H, 7.85%; N, 9.92%.

5.6.3.2 1-(3-pyridyl)-1-(p-methylphenyl)-3-(1-pyrrolidino)propan-1-ol (58)

m.p. 112°C, ^{1}H-NMR 8.73 (s, 1H, Py H-2'), 8.54 (s, 1H, disappears on deuteration), 8.43 (d, 1H, Py H-6'), 7.76 (d, 1H, Py H-4'), 7.35 (d, 2H, A_2 of A_2B_2), 7.21 (t, 1H, Py H-5'), 7.17 (d, 2H, B_2 of A_2B_2), 2.65 (m, 6H $\underline{CH_2}$-CH_2 and pyrrolidino), 2.42 (t, 2H, CH_2-$\underline{CH_2}$), 2.30 (s, 3H, methyl), 1.76 (s, 4H, pyrrolidino).

Found C, 77.4%; H, 8.36%; N, 9.53%. Calculated $C_{19}H_{24}N_2O$ C, 76.99%; H, 8.16%; N, 9.45%.

5.6.4 Preparation of 4-pyridyl intermediates

Once again the compounds were synthesized using the general method of 5.6.2.1 (details in Table 5.8). The 4-bromopyridine used was liberated from its hydrochloride prior to the start of the reaction.

5.6.4.1 1-(4-pyridyl)-1-(phenyl)-3-(1-pyrrolidino)propan-1-ol (59)

m.p. 155.6°C, ^{1}H-NMR 8.63 (broad s, 1H, disappears on deuteration), 8.52 (d, 2H Py H-2' and H-6'), 7.46 (d, 2H, phenyl), 7.41 (d, 2H, Py H-3' and H-5'), 7.31 (t, 2H, phenyl), 7.22 (t, 1H, phenyl), 2.62 (m, 2H, $\underline{CH_2}$-CH_2), 2.54 (s, 4H, pyrrolidino), 2.40 (m, 2H, CH_2-$\underline{CH_2}$N), 1.80 (s, 4H, pyrrolidino).

Found C, 77.0%; H, 7.85%; N, 9.73%. Calculated, $C_{18}H_{22}N_2O$, C, 76.56%; H, 7.85%; N,9.92%.

5.6.4.2 1-(4-pyridyl)-1-(p-methylphenyl)-3-(1-pyrrolidino)propan-1-ol (60)

m.p. 177-179°C, ^{1}H-NMR 8.62 (d, 2H Py H-2' and H-6'), 8.07 (d,2H, Py H-3' and H-5'), 7.31 (d, 2H, A_2 of A_2B_2), 7.20 (d, 2H, B_2 of A_2B_2), 3.63 (m, 2H), 3.0 (m, 4H), 2.20 (s, 3H, methyl) 2.00 (s, 6H, pyrrolidino).

5.6.5 Acid catalysed dehydration of 2-pyridyl, 3-pyridyl and 4-pyridyl propan-1-ols

Dehydration of the alcohols was achieved using 85% H_2SO_4 and a temperature range of 100 - 120°C. Varying lengths of dehydration times at the above condition yielded the different isomers. The oily reaction mixture was poured onto ice/NH_3 and extracted with ether (3 x 100mls). The ether layer was dried ($MgSO_4$) and evaporated under reduced pressure to yield an orange/brown oil. Purification of the dehydrated product so formed was by recrystallisation of the oxalate salt.

The dehydration conditions for the various compounds are given in Table 5.9.

5.6.5.1 E-1-(2-pyridyl)-1-(p-ethylphenyl)-3-pyrrolidino prop-1-ene (40)

m.p. 149-150°C, λ max 229 and 275nm, ^{1}H-NMR 8.52 (d, 1H, Py H-6'), 8.14 (t, 1H, Py H-4'), 7.67 (t, 1H, Py H-5'), 7.58 (d, 1H, Py H-3'), 7.36 (d, 2H, A_2 of A_2B_2), 7.14 (d, 2H, B_2 of A_2B_2), 6.63 (t, 1H, vinylic C<u>H</u>), 3.99 (d, 2H, C<u>H$_2$</u>N),), 3.63 (m, 2H, pyrrolidino), 2.97 (m, 2H, pyrrolidino), 2.65 (q, 2H, Ar-<u>CH$_2$</u>-CH$_3$) 2.00 (s, 4H, pyrrolidino), 1.17 (t, 3H, Ar-CH$_2$-<u>CH$_3$</u>).

HPLC, R_T 20 mins.

Compound No	X	Y	Isomer	Conditions[1]	Purity	Rx [2]
40	2-pyridyl	p-ethyl-phenyl	E	120°C, 1 hour	100%	1
41	p-ethyl-phenyl	2-pyridyl	Z	120°C, 10 mins	92%	3
36	2-pyridyl	phenyl	E	120°C, 4 hours	90%	2
37	phenyl	2-pyridyl	Z	100°C, 10 mins 120°C, 1 hour	60% 68%	3 1
38	2-pyridyl	p-bromo-phenyl	E	120°C 4 hours	100%	3
39	p-bromo-phenyl	2-pyridyl	Z	100°C, 30 mins 100°C, 1 hour	70% 50%	2 0
45	3-pyridyl	phenyl	E	120°C, 30 mins 120°C, 1 hour	90% 91%	2 3
46	3-pyridyl	p-methyl-phenyl	E	120°C, 30 mins	96%	3
49	4-pyridyl	phenyl	E	120°C 4 hours	90%	1
48	phenyl	4-pyridyl	Z	120°C, 30 mins 100°C, 1 hour	100% 100%	3 3

Table 5.9 Experimental conditions for dehydration of propan-1-ols

Footnotes:

1 All dehydrations were carried out using 85% H_2SO_4

2 Rx, means the number of recrystallisations of the oxalate salt from EtOH

5.6.5.2 Z-1-(2-pyridyl)-1-(p-ethylphenyl)-3-pyrrolidino prop-1-ene (41)

λ max 260 nm, ^{1}H-NMR 8.62 (d, 1H, Py H-6'), 8.01 (t, 1H, Py H-4'), 7.66 (t, 1H, Py H-5'), 7.60 (d, 1H, Py H-3'), 7.31 (d, 2H, A_2 of A_2B_2), 7.10 (d, 2H, B_2 of A_2B_2), 6.33 (t, 1H, vinylic C<u>H</u>), 3.83 (d, 2H, C<u>H</u>$_2$N),), 3.61 (m, 2H, pyrrolidino), 2.93 (m, 2H, pyrrolidino), 2.50 (q, 2H, Ar-<u>CH</u>$_2$-CH$_3$) 1.97 (s, 4H, pyrrolidino), 1.06 (t, 3H, Ar-CH$_2$-<u>CH</u>$_3$).

HPLC, R_T 29 mins.

5.6.5.3 E-1-(2-pyridyl)-1-(phenyl)-3-pyrrolidino prop-1-ene (36)

m.p. 166°C, λ max 226.5 and 276.5 nm, ^{1}H-NMR 8.55 (d, 1H, Py H-6'), 8.20 (t, 1H, Py H-4'), 7.68 (m, 2H, Py H-3' and H-5'), 7.58 (m, 3H, phenyl), 7.28 (m, 2H, phenyl), 6.69 (t, 1H, vinylic C<u>H</u>), 4.00 (d, 2H, C<u>H</u>$_2$N),), 3.63 (s, 2H, pyrrolidino), 3.00 (s, 2H, pyrrolidino), 2.01 (s, 4H, pyrrolidino),

HPLC, R_T 8 mins.

5.6.5.4 Z-1-(2-pyridyl)-1-(phenyl)-3-pyrrolidino prop-1-ene (37)

m.p. 166°C, λ max 242 nm, ^{1}H-NMR 8.64 (d, 1H, Py H-6'), 8.08 (t, 1H, Py H-4'), 7.73 (t, 1H, Py H-5'), 7.53 (d, 2H, Py H-3' and phenyl), 7.40 (d, 2H, phenyl), 7.28 (d, 2H, phenyl), 6.38 (t, 1H, vinylic C<u>H</u>), 3.84 (d, 2H, C<u>H</u>$_2$N),), 3.62 (s, 2H, pyrrolidino), 3.00 (s, 2H, pyrrolidino), 2.00 (s, 4H, pyrrolidino),

HPLC, R_T 11 mins.

5.6.5.5 E-1-(2-pyridyl)-1-(p-bromophenyl)-3-pyrrolidino prop-1-ene (38)

m.p. 176 - 178°C, λ max 230 and 275 nm, ^{1}H-NMR 8.50 (d, 1H, Py H-6'), 8.08 (t, 1H, Py H-4'), 7.65 (t, 3H, Py H-5'and A_2 of A_2B_2), 7.56 (d, 1H, Py H-3'), 7.14 (d, 2H, B_2 of A_2B_2), 6.65 (t, 1H, vinylic C<u>H</u>), 3.97 (d, 2H, C<u>H</u>$_2$N),), 3.62 (s, 2H, pyrrolidino), 2.98 (s, 2H, pyrrolidino), 2.00 (s, 4H, pyrrolidino),

HPLC, R_T 24 mins.

Found C, 55.6%; H, 4.86%; N, 6.37%. Calculated $C_{18}H_{19}N_2Br$ C, 55.44%; H, 4.88%; N, 6.47%.

5.6.5.6 Z-1-(2-pyridyl)-1-(p-bromophenyl)-3-pyrrolidino prop-1-ene (39)

m.p. 181 - 182°C, λ max 260 nm, ^{1}H-NMR 8.62 (d, 1H, Py H-6'), 8.08 (t, 1H, Py H-4'), 7.64 (t, 1H, Py H-5'),, 7.44 (m, 3H, Py H-3' and A_2 of A_2B_2), 7.08 (d, 2H, B_2 of A_2B_2), 6.35 (t, 1H, vinylic C<u>H</u>), 3.84 (d, 2H, C<u>H</u>$_2$N),), 3.61 (s, 2H, pyrrolidino), 2.97 (s, 2H, pyrrolidino), 1.98 (s, 4H, pyrrolidino),

HPLC, R_T 32 mins.

5.6.5.7 E-1-(3-pyridyl)-1-(phenyl)-3-pyrrolidino prop-1-ene (45)

m.p. 165 -166°C, λ max 226 and 244 nm, ^{1}H-NMR 8.63 (d, 1H, Py H-6'), 8.58 (s, 1H, Py H-2'), 8.25 (d, 1H, Py H-4'), 7.80 (t, 1H, Py H-5'), 7.53 (d, 3H, phenyl), 7.27 (t, 2H, phenyl), 6.43 (t, 1H, vinylic C<u>H</u>), 3.96 (d, 2H, C<u>H</u>$_2$N),), 3.65 (m, 2H, pyrrolidino), 3.00 (s, 2H, pyrrolidino), 2.01 (s, 4H, pyrrolidino),

HPLC, R_T 7.81 mins (Z-isomer impurity R_T 10.04 mins).

Found C, 68.0%; H, 6.26%; N, 7.90%. Calculated $C_{18}H_{20}N_2$ C, 67.78%; H, 6.21%; N, 7.91%.

5.6.5.8 E-1-(3-pyridyl)-1-(p-methylphenyl)-3-pyrrolidino prop-1-ene (46)

m.p. 194-196°C, λ max 227 nm, ^{1}H-NMR 8.63 (d, 1H, Py H-6'), 8.60 (s, 1H, Py H-2'), 8.22 (d, 1H, Py H-4'), 7.78 (t, 1H, Py H-5'), 7.38 (d, 2H, A_2 of A_2B_2), 7.17 (d, 2H, B_2 of A_2B_2), 6.38 (t, 1H, vinylic C<u>H</u>), 3.97 (d, 2H, C<u>H$_2$</u>N),), 3.64 (s, 2H, pyrrolidino), 3.00 (s, 2H, pyrrolidino), 2.39 (s, 3H, Ar-<u>Me</u>), 2.01 (s, 4H, pyrrolidino).

Found C, 68.9%; H, 6.62%; N, 7.60%. Calculated $C_{19}H_{22}N_2$ C,68.46%; H, 6.57%; N, 7.60%.

5.6.5.9 Z-1-(4-pyridyl)-1-(phenyl)-3-pyrrolidino prop-1-ene (48)

m.p. 189 -191°C, λ max 242 nm, ^{1}H-NMR 8.76 (d, 2H, Py H-2' and H-6'), 7.71 (d, 2H, Py H-3' and H-5'), 7.41 (t, 3H, phenyl), 7.30 (d, 2H, phenyl), 6.41 (t, 1H, vinylic C<u>H</u>), 3.90 (d, 2H, C<u>H$_2$</u>N),), 3.64 (s, 2H, pyrrolidino), 2.99 (s, 2H, pyrrolidino), 2.00 (s, 4H, pyrrolidino),

Found C, 68.1%; H, 6.31%; N, 7.95%. Calculated $C_{18}H_{20}N_2$ C, 67.78%; H, 6.21%; N, 7.91%

5.6.5.10 E-1-(4-pyridyl)-1-(phenyl)-3-pyrrolidino prop-1-ene (49)

^{1}H-NMR 8.67 (d, 2H, Py H-2' and H-6'), 7.93 (d, 2H, Py H-3' and H-5'), 7.56 (t, 3H, phenyl), 7.28 (d, 2H, phenyl), 6.77 (t, 1H, vinylic C<u>H</u>), 3.99 (d, 2H, C<u>H$_2$</u>N),), 3.66 (s, 2H, pyrrolidino), 2.99 (s, 2H, pyrrolidino), 2.00 (s, 4H, pyrrolidino).

Chapter 6

Pharmacological Testing and Discussion of Results

6.1 Introduction

Typical H_1 agonist actions of histamine relate to the contraction of bronchiolar and gastro-intestinal smooth muscle and therefore, H_1 antagonist compounds discussed here characteristically oppose these actions. The methods of assessing the anti-histamine activity of the compounds cited in this thesis are detailed in this chapter.

i) *In-vitro* methods including isolated guinea-pig ileum studies and binding experiments.

ii) *In-vivo* studies using a histamine releasing stimulant administered by i.v. (48/80 lethality test).

6.2 In-vitro Methods

6.2.1 Isolated Guinea Pig Ileum Studies

Inhibition of the contractions of isolated guinea-pig ileum, induced by histamine is the most commonly used *in-vitro* test method for quantitative assessment of antagonist activity. The potency of an antagonist is usually expressed by determination of the pA_2 value ie. the negative logarithm of the dose of antagonist that reduces the effects of a double dose of agonist to that of a single dose (Schild, 1947). Alternatively, log affinity constants (log K_b) are reported (Adamson et al, 1969) - these two parameters are numerically equivalent (ie. $pA_2 = \log K_b$).

Testing Methods

The following results were carried out in the Department of Pharmacology, University of Bristol, under the direction of Dr. R.B. Barlow. The affinity constants were measured on the guinea pig isolated ileum, at 30°C and/or 37°C using an automated apparatus (Edinburgh Staff, 1974). The ileum was bathed in Krebs solution (Edinburgh Staff, 1974) and the organ baths were aerated with 95% oxygen; 5% carbon dioxide. A regular two minute dosing regime was maintained using a Commondore Pet computer and electrical relays to control the drug administration.

Time(s)

0 - Add low dose of agonist

30 - Krebs wash

120 - Add high dose of agonist

150 - Krebs wash

240 - Add low dose

The high dose of agonist was always twice that of the low dose. Control doses were normally 1 & 2 x 10^{-7}M histamine (NB. - both responses should lie on the linear portion of the log dose - response curve). The histamine induced ileum contractions were measured isotonically against a 0.5g load using a transducer and moving chart pen recorder. Exposure of the ileum to control doses was maintained until regular sized responses were produced for a minimum of four pairs of high and low doses.

The antagonist was diluted in the Krebs solution and histamine solutions were made up with this antagonist wash.

The effects of the antagonists were slow in onset and equilibrium was complete only after 15-45 min (depending upon the concentration of the antagonist) when the responses became constant. Approximate dose ratios were chosen for each concentration of antagonist, so that the responses produced by the histamine/antagonist mixture were approximately the same as those of the histamine control solutions.

The dose ratio (ie. the dose in the absence of antagonist against the dose in the presence of antagonist) produced by one particular concentration of antagonist was calculated by comparing the concentration of histamine used in the presence and absence of antagonist and taking into account the actual size of the responses (Edinburgh Staff, 1974).

The affinity constant K_b was calculated from the dose ratio and the concentration of antagonist - according to the Gaddum - Schild equation (Schild, 1947; Schild, 1949).

$$DR = 1 + BK_b$$

Gaddum Schild Equation

(derivation from Barlow, 1980)

DR = Dose ratio

K_b = Affinity constant

B = Antagonist concentration

The graph of DR against concentration (B) should be a straight line with a slope K_b. Since every antagonist was tested at several concentrations, so that the dose ratio ranged from 10-700 - it is more convenient to plot log (DR-1) against log B. This should be a straight line with a slope of 1 and when log (DR-1) = 0, log B = -log K_b.

$$\text{Log } K_b = \log \frac{(DR-1)}{(B)}$$

Fresh solutions of the histamine and antagonist were made up each day - so eliminating any risk of deterioration in the efficacy of these agents.

6.2.2 Results and Discussion

It was found that the responses in the presence of antagonist did not exactly match the standard agonist responses. A correction factor was calculated on a microcomputer using a programme based on the theory of the 4-point bioassay (Edinburgh Staff, 1974).

For each antagonist concentration, the results were averaged to produce a mean dose ratio and mean log K_b. Also calculated was a weighted mean dose ratio and log K_b - this involved the use of a microcomputer to apply a weighting to the difference between the tried dose-ratio and the dose-ratio obtained after correction.

Tables 6.1 to 6.5 show estimates of log K_b ± s.e. at different concentrations of histamine agonist and at the two temperatures ie: 30 and 37°C. (Figures in parentheses show the number of results at that concentration).

Table 6.1: Estimates of log K_b for chlorpheniramine maleate (17c) at different concentrations

(+) Chlorpheniramine maleate

	30°C	37°C
1nM	9.870 ± 0.018 (6)	9.451 ± 0.049 (6)
5nM	9.896 ± 0.039 (5)	9.331 ± 0.027 (5)
10nM	10.123 ± 0.092 (2)	9.381 ± 0.067 (3)
20nM	10.122 ± 0.034 (5)	9.289 ± 0.071 (3)
50nM	-	9.133 ± 0.026 (3)
MEAN	9.975 ± 0.029 (18)	9.339 ± 0.024 (20)

(-) Chlorpheniramine maleate

0.5μM	6.949 ± 0.005 (2)	6.674 ± 0.118 (2)
1μM	6.831 ± 0.028 (4)	6.701 ± 0.063 (4)
5μM	6.642 ± 0.048 (4)	6.383 ± 0.092 (4)
10μM	6.596 ± 0.113 (2)	6.488 ± 0.056 (2)
MEAN	6.749 ± 0.039 (12)	6.55 ± 0.043 (12)

Table 6.2: Estimates of log K_b for brompheniramine maleate (17b) at different concentrations

(+) Brompheniramine maleate

	30°C	37°C
5nM	10.137 ± 0.071 (8)	-
10nM	10.225 ± 0.063 (13)	9.499 ± 0.062 (5)
30nM	10.482 ± 0.068 (9)	9.214 ± 0.074 (6)
50nM	-	9.177 ± 0.079 (3)
100nM	10.396 ± 0.097 (5)	9.258 ± 0.066 (7)
500nM	-	9.092 ± 0.139 (4)
MEAN	10.295 ± 0.023 (35)	9.259 ± 0.257 (25)

(-) Brompheniramine maleate

3μM	7.198 ± 0.101 (3)	6.801 ± 0.054 (3)
10μM	7.081 ± 0.099 (3)	6.568 ± 0.088 (4)
20μM	7.053 ± 0.168 (3)	6.730 ± 0.072 (3)
MEAN	7.111 ± 0.022 (9)	6.687 ± 0.034 (10)

Table 6.3: Estimates of log K_b for carbinoxamine tatrate (9) at different concentrations

(-) Carbinoxamine (dextro L tartrate salt)

	30°C	37°C
30nM	9.174 ± 0.042 (3)	8.796 ± 0.075 (4)
92.3nM	9.065 ± 0.053 (7)	8.762 ± 0.052 (7)
277nM	8.976 ± 0.018 (5)	8.727 ± 0.087 (5)
461nM	8.878 ± 0.042 (5)	8.565 ± 0.063 (5)
1000nM	8.970 ± 0.016 (4)	8.486 ± 0.080 (5)
MEAN	9.005 ± 0.019 (24)	8.670 ± 0.024 (26)

(+) Carbinoxamine (levo D tartrate salt)

0.2μM	7.324 ± 0.023 (5)	7.227 ± 0.025 (5)
3μM	7.161 ± 0.052 (5)	7.103 ± 0.059 (5)
10μM	7.091 ± 0.059 (5)	7.096 ± 0.080 (5)
MEAN	7.192 ± 0.026 (15)	7.142 ± 0.016 (15)

Table 6.4: Estimates of log K_b for mebrophenhydramine (11) maleate at different concentrations

(-) Mebrophenhydramine maleate

	30°C	37°C
2nM	9.983 ± 0.064 (6)	9.794 ± 0.085 (7)
10nM	9.841 ± 0.101 (5)	9.695 ± 0.086 (7)
50nM	10.018 ± 0.128 (5)	9.656 ± 0.112 (7)
MEAN	9.949 ± 0.019 (16)	9.715 ± 0.013 (21)

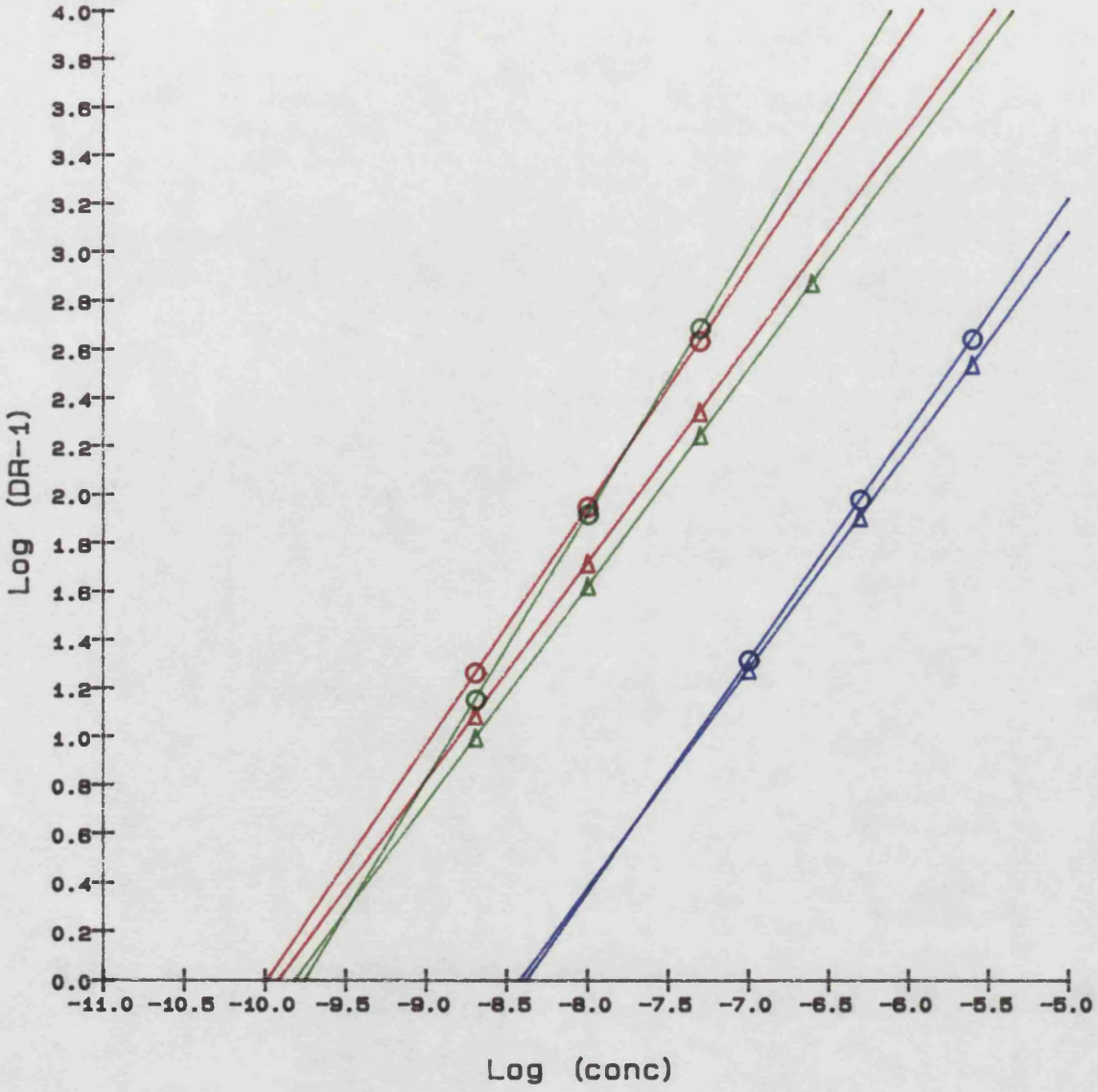
(+) Mebrophenhydramine maleate

0.1μM	8.332 ± 0.090 (5)	8.308 ± 0.056 (5)
0.5μM	8.234 ± 0.099 (5)	8.124 ± 0.066 (5)
2.5μM	8.274 ± 0.111 (5)	8.187 ± 0.068 (5)
MEAN	8.280 ± 0.011 (15)	8.206 ± 0.020 (15)

RS Mebrophenhydramine maleate

2nM	9.866 ± 0.06 (4)	9.745 ± 0.06 (4)
10nM	9.886 ± 0.07 (4)	9.568 ± 0.05 (4)
50nM	10.021 ± 0.10 (3)	9.559 ± 0.03 (4)
0.25μM	-	9.516 ± 0.15 (2)
MEAN	9.916 ± 0.021 (11)	9.609 ± 0.024 (14)

Pharmacological data of (+), (-)
and RS mebrophenhydramine
on guinea pig ileum assay.



30 C (-) mebrophenhydramine
0.982833*X +9.808661
37 C (-) mebrophenhydramine
0.899142*X +8.906466
30 C (+) mebrophenhydramine
0.952074*X +7.977687
37 C (+) mebrophenhydramine
0.903433*X +7.600868
30 C RSmebrophenhydramine
1.097997*X +10.70531
37 C RSmebrophenhydramine
0.896423*X +8.795088

Table 6.5: Estimates of log K_b for triprolidine (23) hydrochloride at different concentrations

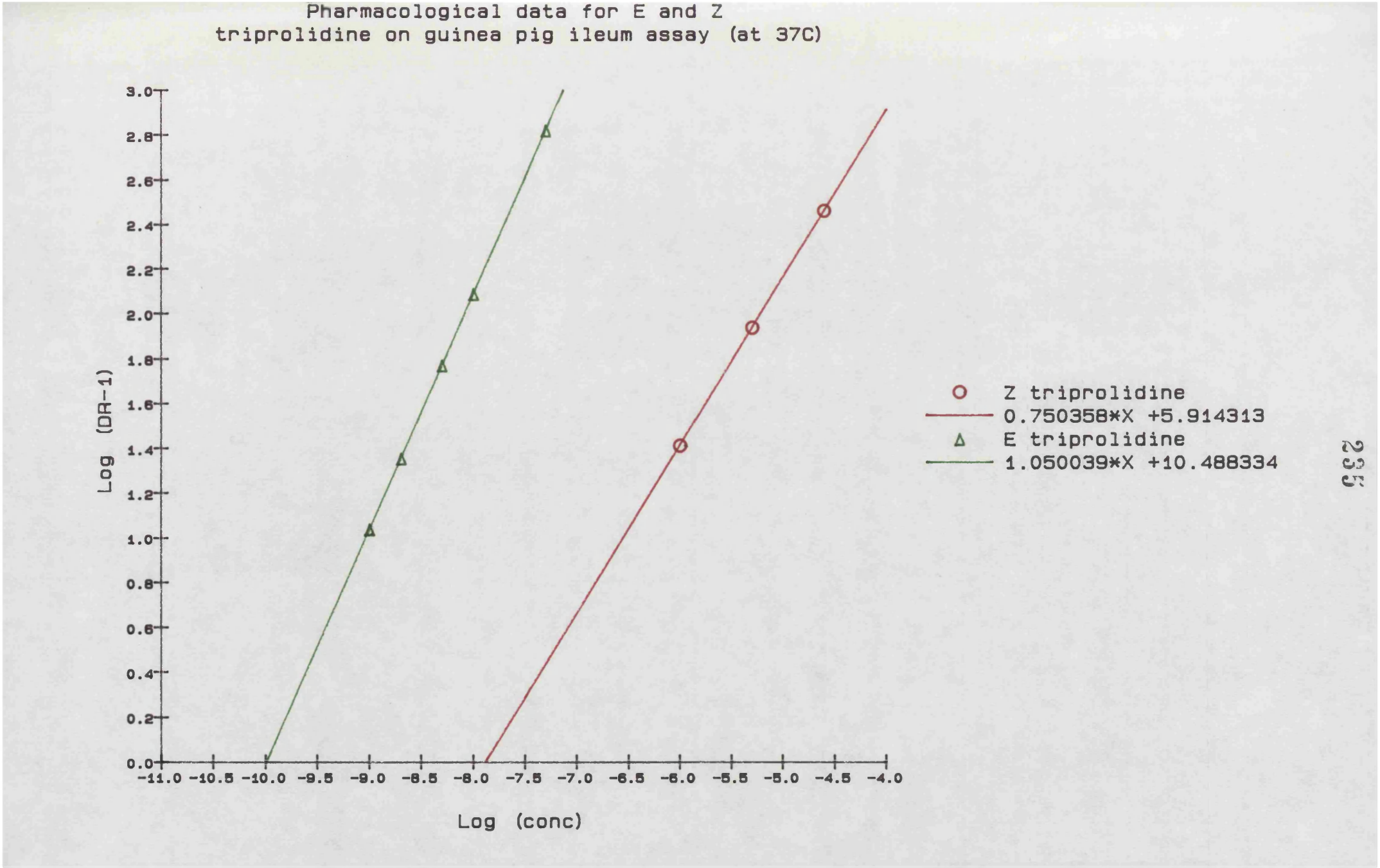
E-triprolidine

37°C

1nM	9.973 ± 0.055 (6)
2nM	10.067 ± 0.027 (4)
5nM	9.982 ± 0.071 (6)
10nM	10.088 ± 0.021 (2)
50nM	10.089 ± 0.081 (8)
MEAN	10.034 ± 0.011 (26)

Z-triprolidine

1μM	7.436 ± 0.022 (7)
5μM	7.195 ± 0.021 (7)
25μM	7.137 ± 0.039 (7)
MEAN	7.256 ± 0.029 (21)


Pharmacological data for E and Z
triprolidine on guinea pig ileum assay (at 37C)
Log (DR-1)
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-11.0 -10.5 -10.0 -9.5 -9.0 -8.5 -8.0 -7.5 -7.0 -6.5 -6.0 -5.5 -5.0 -4.5 -4.0
Log (conc)
Z triprolidine
0.750358*X +5.914313
E triprolidine
1.050039*X +10.488334

6.2.3 Do the Results Fit in with the Gaddum - Schild Equation?

If the results obey the Gaddum-Schild equation

ie. Dose-ratio (DR)= $1 + BK_b$ B = antagonist concentration.
K_b = affinity constant of antagonist for receptor

then the antagonism is a simple competitive process and it is valid to make comparisons of the efficacy. The Schild plot of log [DR-1] against log B should give a straight line with a gradient of 1 and when log [DR-1] = 0 then log B = log K_b.

Applying a t test to the slopes obtained establishes whether there is any significant deviation from unity and is an indication of whether the simple competitive mechanism does apply.
Values of t are given by:-

$$t = \frac{\text{slope} - 1}{\text{standard error of slope}}$$

This may be compared to probability values for t for n-2 degrees of freedom. The threshold for significance was taken as the value of t for P(0.05) ie. less than 1 in 20 chance of obtaining the observed slope.

The results are shown in table 6.6.

Table 6.6: Stastistical analysis of the Schild plots of the results

Compound	Temp. °C	Slope (± se)	No. points	t	P(0.05) for n-2 df.	Significantly different from unity
(+) Chlorpheniramine	30	1.189 ± 0.091	4	2.08	4.30	NO
	37	0.800 ± 0.064	5	3.13	3.18	NO
(-) Chlorpheniramine	30	0.721 ± 0.031	4	9.00	4.30	YES
	37	0.800 ± 0.095	4	2.11	4.30	NO
(+) Brompheniramine	30	1.232 ± 0.111	4	2.09	4.30	NO
	37	0.739 ± 0.104	4	2.51	4.30	NO
(-) Brompheniramine	30	0.833 ± 0.071	3	2.35	12.7	NO
	37	0.862 ± 0.243	3	0.57	12.7	NO
(-) Carbinoxamine	30	0.711 ± 0.065	3	4.45	12.7	NO
	37	0.712 ± 0.205	3	1.40	12.7	NO
(+) Carbinoxamine	30	0.872 ± 0.001	3	12.8	12.7	YES
	37	0.925 ± 0.027	3	2.78	12.7	NO
(-) Mebrophenhydramine	30	0.983 ± 0.14	3	0.12	12.7	NO
	37	0.899 ± 0.02	3	5.05	12.7	NO
(+) Mebrophenydramine	30	0.952 ± 0.06	3	0.80	12.7	NO
	37	0.903 ± 0.10	3	0.97	12.7	NO
RS Mebrophenhydramine	30	1.098 ± 0.05	3	1.96	12.7	NO
	37	0.896 ± 0.04	4	2.60	4.30	NO
E-Triprolidine	37	1.050 ± 0.04	5	1.25	3.18	NO
Z-Triprolidine	37	0.750 ± 0.09	3	2.79	12.7	NO

From the t tests on the slopes in the Schild plots - it is not possible to conclude that the compounds antagonistic action is purely competitive; similarly, it is not possible to prove that they are not. Better information on this would have been obtained from improved experimental design eg. a wider range of antagonist concentrations would have given a more precise extrapolated value of log K and would have led to a better indication of the slope of the Schild plot.

6.2.4 Changes in Log K with Temperature

Table 6.7 shows the change in log K_b (using mean log K_b) with temperature for each enantiomer. The figures are given for 30°C relative to 37°C

ie. antilog [Mean log K at 30°C - mean log K at 37°C].

The results show that the more active enantiomer is more sensitive to temperature rise than the less active isomer and that greater antagonism is produced at 30°C than at 37°C ie. stereospecificity is inversely related to temperature. This increased binding of the antagonists at lower temperatures provides information about the enthalpy of reaction between drug and receptor. These effects may be quantified in terms of the enthalpy (internal energy) change for the reaction (Δ H) (Table 6.8):-

$$\Delta H = -\frac{R\ \Delta \ln K}{\Delta\ 1/T}$$

ΔH = Enthalpy change

$R = 8.314$ joules deg $^{-1}$ mol $^{-1}$

$\Delta 1/T = 7.45 \times 10^{-5}$

Table 6.7: Change in Log K_b with temperature and enthalpy change (ΔH).

Compound	Difference Mean Log K	Ratio 30 : 37	ΔH KJ mol^{-1}
(+) Chlorpheniramine	0.636	4.33	-164
(-) Chlorpheniramine	0.194	1.56	-50
(+) Brompheniramine	1.036	10.86	-266
(-) Brompheniramine	0.424	2.66	-108
(-) Carbinoxamine	0.335	2.16	-86
(+) Carbinoxamine	0.050	1.12	-12.9
(-) Mebrophenhydramine	0.234	1.71	-60
(+) Mebrophenhydramine	0.074	1.19	-19

In all cases the sign is negative indicating that the drug receptor interaction is an exothermic process and binding is greater at lower temperatures. Studies of this type are confined in the range of temperatures used because of the limits in the performance of the biological preparations.

6.2.5 Differences in activity of enantiomeric pairs

Comparison of activity between enantiomers may be made from the ratio of affinity constants ie. the antilog of the difference between the log K_bs of the enantiomeric pair (Table 6.8). There are two possible methods of calculating the value of log K for each enantiomer at each temperature. The first involves taking the mean log K for all values obtained over all concentrations and the second by extrapolation of the Schild plot to where log DR - 1 =0. This latter method is limited by the spread of the results and since this is rather wide in some cases, this method has not been employed as such these results have not been quoted.

These results have shown that the log K_b values for (+) chlorpheniramine (S-configuration) and (+) brompheniramine (S) are greater than those for the corresponding levo isomers. Similarly (-) - carbinoxamine (S) and (-) mebrophenhydramine (R-configuration from earlier circular dichroism studies) have larger log K_b values than their corresponding dextro isomers.

Table 6.8: The ratio of the enantiomeric and geometric pairs using mean log K_b data

	Temp. °C	(+) Mean Log K ± (S.E.)	(-) Mean Log K ± (S.E.)	Difference	Ratio of activity
Chlorpheniramine	37	9.339 ± 0.024 (20)	6.555 ± 0.043 (12)	2.784	608
	30	9.975 ± 0.029 (18)	6.749 ± 0.039 (12)	3.226	1683
Brompheniramine	37	9.259 ± 0.027 (25)	6.687 ± 0.034 (10)	2.572	373
	30	10.295 ± 0.023	7.111 ± 0.022 (9)	3.184	1528
		(-)	(+)		
Carbinoxamine	37	8.670 ± 0.024 (26)	7.142 ± 0.016 (15)	1.528	34
	30	9.005 ± 0.019 (24)	7.192 ± 0.026 (15)	1.813	65
Mebrophenhydramine	37	9.715 ± 0.013 (21)	8.206 ± 0.020 (15)	1.509	32
	30	9.949 ± 0.019 (16)	8.280 ± 0.011 (15)	1.669	47
		E	Z		
Triprolidine	37	10.034 ± 0.011 (26)	7.256 ± 0.029 (21)	2.778	600

6.2.6 In vitro studies with dimethindene (20)

These experiments were carried out in the Pharmacology Department of Smith Kline & French Research Ltd and the results quoted are against histamine stimulated contraction of guinea pig ileum after 8 minute equilibrium at 30°C (Table 6.9).

Isomer	pA_2	(95% limits)	Slope of Schild plot	N
(+) dimethindene (-) tartrate	7.86	(7.71 - 8.07)	0.74 ± 0.19	3
(-) dimethindene (+) tartrate	9.54	(9.31 - 9.83)	0.73 ± 0.33	4

Table 6.9 pA_2 values for the two isomers of dimethindene

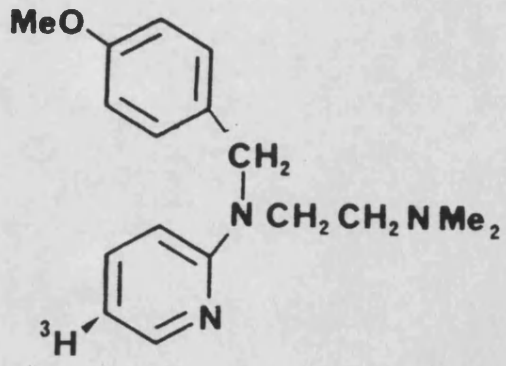
The results show that (-) dimethindene ((+) tartrate) is more active than its dextro isomer, results in agreement with Borchard et al (1985) who published pA_2 values (on guinea pig ileum) of 9.1 for the levo isomer and 7.8 for the dextro. The stereospecific index for these two isomers is approximately 50 for the SKF data and 20 from the 1985 report.

6.3 BINDING STUDIES

6.3.1 Introduction

Direct study of the binding of antagonist ligand to H_1-receptors is now a procedure of increasing application. (Review, Hill, 1987). The procedure involves measuring displacement of radiolabelled (^3H) mepyramine from the membrane fraction of guinea pig brain deemed to contain a high population of H_1-histamine receptors. Irrelevant contributions due to non-specific uptake of the radio-ligand are established by carrying out the binding experiment in the presence of a large excess of 'cold' ligand.

Mepyramine (6) is the compound which has been principally used as a probe for H_1-receptor responses in both central and peripheral tissues because of its high affinity and relative selectivity.



(6)

The selectivity of mepyramine and other compounds for the H_1-receptor is much dependent upon the concentration used. Thus, although, mepyramine can be considered to act selectivity on H_1-receptors in the concentration range 1-100nM, at higher concentrations it will begin to antagonize muscarinic and H_1-receptors also.

Hill et al (1978) found that the affinity constant for (+) chlorpheniramine ($1.2 \times 10^9 M^{-1}$) was over 200 times greater than that of the levo isomer ($5 \times 10^6 M^{-1}$) and that these values closely resembled those measured at guinea pig ileum sites. Chang et al (1979) also compared the binding affinities of (+) and (-) chlorpheniramine and large differences between antipodes were found for a variety of mammalian brains (Table 6.10).

	K_i values[1] for binding to mammalian brains				
	Human	Guinea -pig	Rat	Rabbit	Monkey
(+) chlorpheniramine	4.2±0.7	1.4±0.8	8.0±2.7	21	9.1
(-) chlorpheniramine	350±170	130±50	700±110	2100	730

Table 6.10: K_i values for binding of chlorpheniramine isomers to mammalian brains

Footnotes:

1. K_i (nM) $= \dfrac{IC_{50}}{(1 + [^3H]\ \text{mepyramine})K_D}$

1nM for guinea pig and 2nM for rest.

Non-specific binding in the presence of 2nM triprolidine

K_D - dissociation constant from Scatchard plot.

IC_{50} - antipodal concentration that displaces 50% of the specifically bound radioligand.

6.3.2 Binding Study Results

The following binding study results, using [^{3}H] mepyramine were measured by Dr M. Young, Department of Pharmacology at the University of Cambridge.

Dimethindene (tartrate)

Three measurements were made on each dimethindene isomer (as tartrate salts) using guinea pig cerebellar membranes and one measurement of each on a digitonin solubilized preparation. The combined results were as follows (Table 6.11).

	$K_a(M^{-1})$ (± s.e.)		
	(-)dimethindene tartrate	(+)dimethindene tartrate	Ratio
Membrane Bound	5.05 ±0.15 X10^9	2.21 ±0.09 X10^7	229 ±12
Soluble	2.20 ±0.39 X10^9	9.45 ±1.10 X10^6	233 ±49

Table 6.11 Affinity constants for the two isomers of dimethindene

Triprolidine and its analogues

The affinity constants quoted below (Table 6.12) for the triprolidine and its Z isomer were measured on a particulate fraction of guinea pig cerebellum. The first results were obtained for samples of triprolidine and its isomer known to contain impurities of the minor isomer. The latter results (3 in total) are for isomerically pure (by HPLC) samples of each isomer.

E-Isomer $K_a(M^{-1})$(Hill coefficient)	Z-Isomer $K_a(M^{-1})$(Hill coefficient)
$1.2 \pm 0.1 \times 10^9$ (1.09 ±0.05)	$6.2 \pm 0.3 \times 10^7$ (0.94 ±0.05)
$1.9 \pm 0.1 \times 10^9$ (1.00 ±0.03)	$2.0 \pm 0.2 \times 10^7$ (0.95 ±0.07)
$2.3 \pm 0.2 \times 10^9$ (0.95 ±0.06)	$1.7 \pm 0.2 \times 10^7$ (1.08 ±0.09)
$2.3 \pm 0.1 \times 10^9$ (1.02 ±0.06)*	$2.0 \pm 0.1 \times 10^7$ (1.19 ±0.05)*

* guinea pig cerebral cortex

Table 6.12 Affinity constants for the two isomers of triprolidine

Each pair of results were measured on the same homogenate, in the same experiment. These results show a potency ratio of 100, results in agreement with those obtained using guinea pig ileum tests.

The following measurements on the compounds synthesized for this thesis (Chapter 5) were made on homogenates of guinea pig cerebral cortex. The results are shown in Table 6.13.

No	Compound Analogue of Triprolidine	Affinity Constant (No of experiments)
40	E-p-ethylphenyl	$1.84 \pm 0.16 \times 10^9$
38	E-p-bromophenyl	$6.61 \pm 0.54 \times 10^8$
24	E-p-chlorophenyl	$8.99 \pm 0.61 \times 10^8$
34	Z-p-chlorophenyl	$3.95 \pm 0.18 \times 10^7$
48	Z 4-pyridylphenyl	$4.52 \pm 1.27 \times 10^7$

Table 6.13 Affinity constants for synthesized analogues of triprolidine

It is unfortunate that insufficient quantities of purified samples of each isomer were available for testing. It can be seen from Table 6.13, however, that the E isomer of the 2 - pyridyl analogues of triprolidine show high affinity constants (10^8 - 10^9) while the Z isomers tested (34 and 48) show values of the same magnitude to each other but lower than the E isomers. These results are in agreement with those previously quoted for triprolidine and its Z-isomer.

Using the same conditions, the affinity constants for the isomers of mebrophenhydramine were measured. The results (Table 6.14) were in agreement with those calculated from guinea pig ileum assay (Table 6.4).

Isomer	Affinity constant
(+) Mebrophenhydramine maleate	$9.80 \pm 0.06 \times 10^7$ (2)
(-) Mebrophenhydramine maleate	$3.58 \pm 0.30 \times 10^9$ (3)
	$3.46 \pm 0.21 \times 10^9$ (2)

Table 6.14 Affinity constants for the isomers of mebrophenhydramine

Binding studies were also measured at the Schwartz laboratory in Paris. The compounds, dimethindene and mebrophenhydramine, were tested as binding ligands on membranes derived from guinea pig cerebellum and auricle against ^{125}[I] iodobolpyramine. The results from these studies are given in Table 6.15.

Compound	K_i (nM)[1] cerebellum[2]	auricle[3]
(-)dimethindene	0.028 ±0.009	0.05 ±0.01
(+)dimethindene	14.4 ±1.8	7.9 ±2.7
(+)mebrophenhydramine	0.58 ±0.03	1.42 ±0.35
(-)mebrophenhydramine	0.27 ±0.02	0.33±0.04

Table 6.15 K_i values for binding to guinea pig cerebellum and auricle

Footnotes for Table 6.14:

1. $K_i\ (nM) = \frac{IC_{50}}{(1 + [^3H]\ \text{mepyramine})K_D}$
2. 0.16nM ^{125}[I] iodobolpyramine
3. 0.08nM ^{125}[I] iodobolpyramine

It is interesting to note that (-)dimethindene is much more potent than its (+) enantiomer, while there is not much stereoselective separation for the enantiomers of mebrophenhydramine (cf gut bath experiments).

6.4 In-vivo test methods

The most common *in vivo* assays of antihistamines are those in which the abilities of test compounds to protect guinea pigs against lethal doses of i.v. histamine or bronchospasm produced by inhalation of a histamine aerosol are measured (Silva and Antonio, 1978).

The *in vivo* testing carried out by Janssen Pharmaceutica on the resolved and synthesized compounds cited in this thesis involved the use of compound 48/80 to initiate histamine release.

Compound 48/80 is a mixture of oligomers obtained by condensation of *p*-methoxy-N-methyl phenethylamine and formaldehyde (Neimegeer et al, 1978). It is recognised as a potent histamine releasing agent (Dews et al, 1953; Paton, 1951; West, 1958). Compound 48/80 is injected at a challenge dose of 0.5mg/kg and the test compounds are administered s.c. at a dose of 10mg/kg. The test compounds are assumed to be active if the animals have a survival time of >240 minutes.

Table 6.16 shows the results of the 48/80 lethality test for a selection of the triprolidine analogues (Chapter 5) and indicates the number of rats that survived. Each compound and dose of compound was tested on two rats.

Compound	mg/kg s.c. 10	5	2.5	1.5	0.63
E-triprolidine (23)	2	-	2	-	1
E-p-ethyl analogue (37)	2	-	-	1	-
E-p-bromo analogue (36)	2	0	0	-	-
E-p-chloro analogue (24)	2	1	0	-	-
Z-p-chloro analogue (34)	0	-	0	-	-
E-p-methyl-3-pyridyl analogue (46)	2	2	1	-	-
Z-phenyl-4-pyridyl analogue (49)	1	-	0	-	-

Table 6.16 Number of rats (out of 2 tested) that survived the 48/80 lethality test.

6.5 Central Effects of Chiral Antihistamines

6.5.1 Study of the possible central nervous system (CNS) effects of (+) and (-) dimethindene in mice

In vitro studies, on guinea pig ileum, of dimethidene have shown a potency ratio of >50 (-):(+) (Borchard et el, 1985; 6.2.6 of this thesis). Binding study results (6.3.2) show a 200 fold difference in potency of (-) against (+). Tests designed to establish any differences in the sedative properties of the two enantiomers were performed in the mouse (in the Pharmacology Department of Smith Kline and French Research Limited).

The doses of test drug as tartrate salts ((-) 0.5, 5 and 50 mg/kg and (+) 50 mg/kg) and placebo were administered subcutaneously. The CNS effects were assessed by a number of different tests involving behavioural studies and exploratory avtivity. The results of this study were most disappointing and no significant difference was observed between the behavioural effects of (-) and (+) dimethindene.

6.5.2 Alertness and Performance in Man

Under the supervision of Group Captain A.N. Nicholson, at the Royal Air Force Institute of Aviation Medicine, Farnborough, the individual isomers of chlorpheniramine and dimethindene, were administered to human volunteers and their effects on alertness and performance monitored by various tests and compared with a placebo and an active control (triprolidine).

Doses of 10mg (+) and (-) chlorpheniramine maleate, 5mg (+) and (-) dimethindene maleate, 5mg triprolidine hydrochloride and 2 placebos were administered (orally, as a triturate with lactose and enclosed in a gelatin capsule) on separate occasions to 6 healthy volunteers. Three tests (sleep latency, subjective sleepiness and digit symbol substitution) were carried out 1 hour before and ½, 1½ and 3 hours after ingestion.

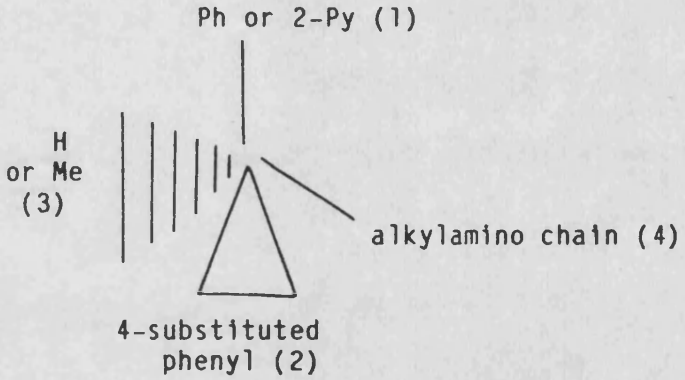
Differences between changes in measures for the tests from before to after ingestion were analysed between enantiomers and between drugs and placebo. No differences were seen ½ hour after ingestion. One and a half hours after ingestion reductions in sleep latencies were greater with (+) chlorpheniramine and (-) dimethindene when compared to placebo. Increased subjective sleepiness was greater with (+) chlorpheniramine than with (-) and placebo (after 1½ and 3 hours) and with (-) dimethindene compared to the (+) isomer (3 hours after ingestion).(+) Chlorpheniramine also showed greater impairment of performance than the (-) isomer (1½ and 3 hours after ingestion).

The importance of these findings, that (+) chlorpheniramine and (-) dimethindene (the active enantiomers <u>in vitro</u> and in binding studies) show increased drowsiness and impairment of performance over their inactive isomers is that it provides a clear indication that sedation arises from central H_1 receptor blockade alone.

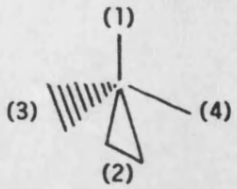
6.5. Discussion

The data on dissymmetric antihistamines of both chiral and geometrical type presented in this chapter and in the Introduction to this thesis, provide evidence of the stereoselective nature of H_1-histamine receptors especially with respect to ligands that block these sites. This work has established the importance of a chiral centre close to the diaryl unit of the structure as opposed to further removed chiral features as is the case for the clemastines (12), phenothiazines and isothipendyl (19).

A large number of pairs of enantiomers with chemically related chiral centres have now been examined, for example the pheniramines (17) and the diphenhydramine-types (7-12) and these results demonstrate that the H_1 receptor is preferentially blocked by a ligand of the general geometry (61). Details for evidence of this are summarized in Table 6.15.



(61)



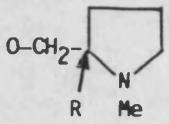
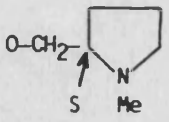
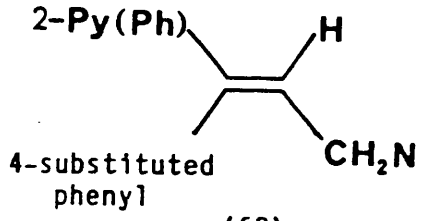
Name and configuration of the more potent enantiomer	Substituent about chiral centre 1	2	3	4	References to Pharmacology
R(+) neobenodine Configuration: Nauta and Rekker, 1978 CD evidence (2.6.4)	Ph	4-MeC_6H_4	H	$O(CH_2)_2NMe_2$	Jarrousse and Regnier, 1951 Nauta and Rekker, 1978
S(-)carbinoxamine Configuration: Barough et al, 1971, CD evidence (2.6.4)	2-Py	4-ClC_6H_4	H	$O(CH_2)_2NMe_2$	Roszkowski and Govier, 1959 Section 6.2.1.
RR(+)clemastine	Ph	4-ClC_6H_4	Me		Ebnother and Weber, 1976
RS(-)diastereomer Configuration: Ebnother and Weber, 1976 CD evidence (2.6.4)	Ph	4-ClC_6H_4	Me		Nauta and Rekker, 1978
R(-)mebrophenhydramine Configuration: CD evidence (2.6.4)	Ph	4-BrC_6H_4	Me	$O(CH_2)_2NMe_2$	Sections 6.2.1., 6.3.1 and 6.4.1.
S(+)pheniramine and 4-Br and 4-Cl analogues Configuration: Shafi'ee and Hite, 1969 CD evidence (2.6.3)	Py	4-XC_6H_4 (X= H, Br Cl)	H	$(CH_2)_2NMe_2$	Roth and Govier, 1958 Brittain et al, 1959 Nauta and Rekker, 1978 Sections 6.2.1 and 6.3.1

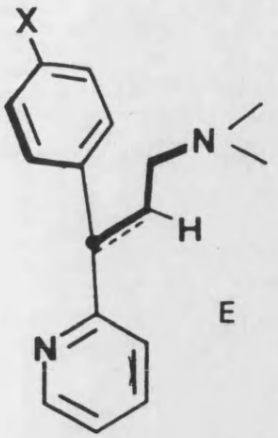
Table 6.17 Review of general geometry for H_1 antihistaminic activity

Receptor sensitivity to the positioning of the two aryl groups around a benzylic carbon is also apparent in antihistamines of the aminopropene type e.g.triprolidine (23). Isomers of the type (62) of the configuration E (for the 2-pyridyl group) have much greater affinities than their corresponding Z isomer (Section 6.2.1 and 6.4.1 of this thesis; Adamson et al, 1951; Ison et al, 1973). These receptor stereoselectivities are maintained in the less potent 3-pyridyl analogues (Section 6.4.1; Hall and Ogren, 1984).

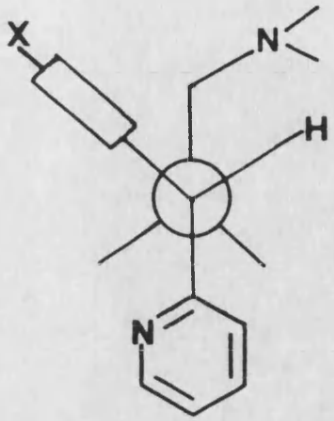


(62)

It is significant that chiral and geometrical configurational relationships correspond. For example, the enantiomeric pheniramines that correspond to the arrangement (A) of triprolidine have the configuration (B) i.e. S the (+) isomer, while their mirror images (C) are equivalent to the feebly active Z analogue of the aminopropene (Scheme 1).

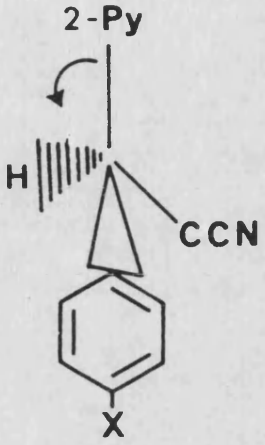


(A)

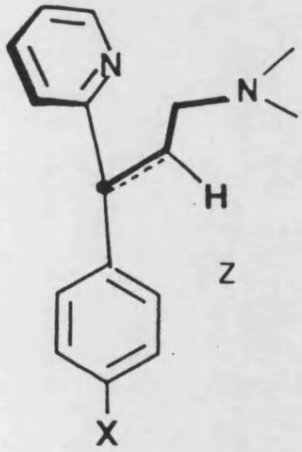


(B)

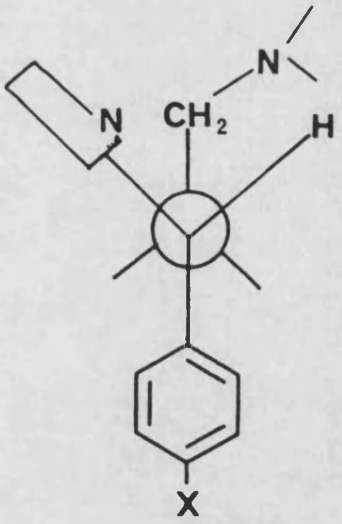
≡



S(+)

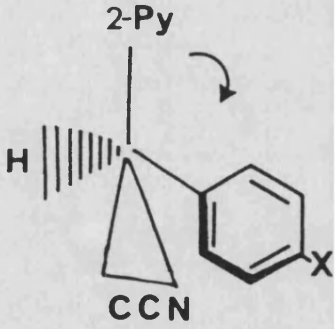


(D)



(C)

≡



R(−)

Scheme 1

From the study of the structural features of many H_1 receptor antagonists it is evident that ligand receptor interactions involve both the aromatic and protonated amino features of the molecules. Of the two receptor sites which accommodate the aromatic groups, one prefers unsubstituted phenyl or 2-pyridyl and the other a para-substituted group. It also follows that the anionic site of the receptor (which associates with the protonated amino feature (N^+H) of the ligand) is closer to the more extended aromatic recognition region (which associates with the para substituted group) i.e. A and B of Scheme 1.

Isomeric potency and affinity ratios

In the absence of isomeric purity it is not advisable to attach too much significance to numerical differences between potency or affinity ratios since false or misleading activity values can result if the isomeric sample is contaminated even with only small amounts of the other isomer. This is most serious when the the less active form is contaminated with the more active isomer.

The optical purities of most chiral anti-histamines examined, especially those prior to 1980, are based on measurements of optical rotation (Chapter 2). As has already been shown in this thesis, this method of measuring optical purity is not absolute and can therefore be misleading.

More recently chromatographic (Souter, 1985; Lochmuller and Souter, 1975; Wainer and Doyle, 1984; Karnes and Sarkar, 1987) and NMR methods (Casy and Mercer, 1988; Mercer, 1988) which quantitate each enantiomer separately are being used to measure optical purity and as such a much greater degree of confidence can be placed on potency ratios of enantiomeric pairs analysed in this way.

From isomeric pairs prepared at Bath and analysed by HPLC and NMR procedures it was found that isomeric activity ratios for molecules that differ in 2-pyridyl and 4-substituted phenyl orientation were substantially greater than those of molecules which involve different dispositions of phenyl and 4-substituted phenyl (Table 6.15). For example the isomeric ratios of activity for brompheniramine at 30°C is 1524 whereas for mebrophenhydramine it is only 47. This trend is also seen in the case of analogues of triprolidine.

This result seems reasonable when one considers that receptor recognition and differentiation of two aromatic units must be the chief factor responsible for affinity differences between isomers (many antihistamines e.g. diphenhydramine (7) the two aryl groups are identical). The greater the difference in aromatic nature e.g. homo vs heteroaryl or homo vs homoaryl, the greater the degree of receptor discrimination between the two aromatic features to be expected.

Of the compounds tested those containing an ether link to the side chain showed lower stereospecificity than those without such a linkage. For example, the introduction of the ether linkage into the chlorpheniramine structure i.e. carbinoxamine produced a reduction in the isomeric potency ratio (at 30°C) from 1683 to 65 (Table 6.9) .

The presence of the oxygen within the side chain generates a more flexible structure and therefore these compounds e.g. carbinoxamine are more able to distort their structure to be accomodated by the histamine receptor unlike the rigid pheniramines. This flexibility factor may also be contributing to the large differences seen between brompheniramine and mebrophenhydramine (as already discussed). In order to evaluate this effect fully a comparison should be made between for example a pheniramine type structure and a non-pyridyl containing analogue.

Chapter 7

References

Abramson, F.B., Barlow, R.B., Mustafa, M.G. and Stephenson, R.P., 1969, Br. J. Pharmacol., 37, 207.

Abrahamsson, A., Carlsson, A., Carnmalm, B. and Dahlen, B., 1976, Acta Chem. Scand. A., 30, 609.

Adamson, D.W. and Billinghurst, J.W., 1950, J. Chem. Soc., 1039.

Adamson, D.W., Barrett, P.A., Billinghurst, J.W. and Jones,T.S.G., 1957, ibid, 2315.

Adamson, D.W., Barrett, P.A., Billinghurst, J.W., Green, A.F. and Jones,T.S.G., 1951, Nature, 168, 204.

Albert, A., Selective Toxicity, Ed 4, Methuen, London, 1968, 384.

Allenmark, S., Bomgren, B and Boren, H., 1983, J. Chrom., 264, 63.

Allenmark, S., Bomgren, B and Boren, H., 1984, ibid., 316, 617.

Alston, D.A., Lilley, T.H. and Stoddart, J.F., 1985, J. Chem. Soc. Chem. Commun., 1600.

Anderson, F. and Bundgaard, H., 1984[1], Int. J. Pharm., 19, 189.

Anderson, F. and Bundgaard, H., 1984[2], Int. J. Pharm., 20, 155.

Arrang, J-M., Garbarg, M., and Schwartz, J-C., 1983, Nature, 302, 832

Arrang, J-M., Garbarg, M., Lancelot, J-C., Lecomte, J-M., Pollard, H., Robba, M., Schunack, W. and Schwartz, J-C., 1988, Nature, 327, 117.

Arrang, J-M., Schwartz, J-C., and Schunack, W., 1985, Eur. J. Pharmacol., 117, 109.

Armstrong, D.W., 1984, J. Liq. Chrom., 7(S-2), 353.

Armstrong, D.W. and De Mond, W., 1984, J. Chrom. Sci., 22, 411.

Armstrong, D.W., Ward, T.J., Armstrong, R.D. and Beesley, T.E., 1986, Science, 232, 1132.

Armstrong, R.D., Ward, T.J., Pattabiraman, N., Benz, C. and Armstrong, D.W., 1987, J. Chrom., 414, 192.

Armstrong, D.W., De Mond, W., Alak, A., Hinze, W.L., Reihl, T.E. and Bui, K.H., 1985, Anal. Chem., 57, 234.

Ash, A.S. and Schild, H.O., 1966, Br. J. Pharmacol., 27, 427.

Astec Informer, 1987, Vol 6, (Advanced Separation Technologies Inc)

Augstein, J., Ham, A.L. and Leeming, P.C., 1972, J. Med. Chem, 15, 466

Barlow, R.B., 1980, Quantitative Aspects of Chemical Pharmacology, Croom Helm, London.

Barrett, W.T., Rutledge, R., Deitrich, A. and Plumer, A.J., 1960, Fed. Proc, 19(1), 210.

Barough, V., Dall, H., Patel, D. and Hite, G., 1971, J. Med. Chem., 14 No 9, 834.

Beckett, A.H. and Casy, A.F., 1955, J. Pharm. Pharmacol., 7, 433.

Beesley, T.E., 1985, Am. Lab., 17, 78.

Beesley, T.E., 1987, personal communication, Guildford.

Benn, R. and Gunther, H., 1983, Angew. Chem. Int. Ed. Eng., 22, 350.

Bijvoet, J.M., Peerdman, A.F. and Van Bommel, A.J., 1951, Nature, 168, 271.

Borchard, U., Hafner, D., Heise, R., 1985, Naunyn-Schmeid Arch. Pharmacol., 330 (suppl) R9, Abstr. 42.

Branch, S.K., Casy, A.F., Hussain, R. and Upton, C., 1988, J. Pharm Pharmacol, 40, 83

Brittain, R.T., D'Arcy, P.F. and Hunt, J.H., 1959, Nature, 183, 734.

Cahn, R.S., Ingold, C.K. and Prelog, V., 1956, Experientia, 12, 81.

Casy,A.F., 1978, Handbook of Experimental Pharmacology, Vol XVIII/2, Histamine II and Antihistamines, Silva, M.R.e., Ed., Berlin, Springer Verlag, 175 and references there cited.

Casy, A.F., 1970, Medicinal Chemistry, Third Edition, Part 1, Edited by Alfred Burger, John Wiley and Sons.

Casy, A.F.and Ison R.R., 1970, J. Pharm. Pharmacol., 22, 270

Casy, A.F. and Mercer, A.D., 1988, Magnetic Resonance in Chemistry, 26, 765.

Chang, R.S.L., Tran, V.T. and Snyder, S.H., 1979, J. Neurochem., 32, 1653.

Crabbe, P.L., 1965, Optical Rotary Dispersion and Circular Dichroism in Organic Chemistry, Holden Day, San Francisco.

Dalgliesh, C.E., 1952, J. Chem. Soc., 137, 3940.

Dappen, R., Arm, H. and Meyer, V.R., 1986, J. Chrom. (Chrom Rev), 373, 1.

De Bruyn, A., Anteunis, M. and Verhegge, G., 1975, Acta. Ciencia Indica, 1 No 2, 83.

Dews, P.B., Wnuck, A.L., Fanelli, R.V., Light, A.E., Tornaben, J.A., Norton, S., ELLIS, C.H. and De Beer, E.J., 1953, J. Pharmacol. Exp. Ther., 107, 1.

Dobashi, A. and Hara, S., 1983, Anal. Chem., 55, 1805.

Drake, A.F., 1986[1], J.Phys. E. Sci. Instrum., 19, 170, (Review).

Drake, A.F., 1986[2], European Spectroscopy News, 69, 10.

Elz, Von S. and Schunack, W., Arzneim. Forsch, 1988, 38, 327

Ebnother, A. and Weber, H.P., 1976, Helv. Chem. Acta., 59, 2462.

Edinburgh Staff, 1974, Pharmacological Experiments on Isolated Preparations, 2nd Ed., Livingstone, Edinburgh.

Fourneau, E. and Bovet, D., 1933, Arch. Int. Pharmacodyn., 46, 179.

Fujioka, K., Kurosaki, Y., Sato, S., Noguchi, T., Noguchi, T. and Yamahira, Y., 1983, Chem. Pharm. Bull., 31, 2416.

Gaddum, 1937, J. Physiol., 89, 7P.

Ganellin, C.R., 1982, Pharmacology of Histamine Receptors, Ganellin, C.R. and Parsons, M.E., Eds, Wright PSG, Bristol, 10.

Gil-Av, E., Tishbee, A. and Hare, P.E., 1980, J. Amer. Chem. Soc., 102, 5115.

Gilman, H. and Spatz, S., 1951, J. Org. Chem., 16, 1485.

Greatbanks, D. and Pickford, R., 1987, Magn. Reson. Chem., 25, 208.

Hall, H. and Ogren, S.O., 1984, Life Sciences, 34, 597.

Herman, D.P., Field, L.R. and Abbott, S., 1981, J. Chrom. Sci., 19, 470.

Hermansson, J., 1983, J. Chrom., 269, 71.

Hermansson, J., 1984, ibid., 298, 67.

Hermansson, J., 1985, ibid., 325, 379.

Hermansson, J. and Eriksson, M., 1986, J. Liq. Chrom., 9(2+3), 621.

Hill, S.J., Emson, P.C. and Young, J.M., 1978, J. Neurochem., 31, 997.

Hinze, W.L., Reihl, T.E., Armstrong, D.W., De Mond, W., Alak, A. and Ward, T., 1985, Anal. Chem., 57, 237.

Hunt, J.H., 1961, J. Chem. Soc., 2228.

Huebner, C.F., Donogue, E., Wenk, P., Sury, E. and Nelson, J.A., 1960, J. Amer. Chem. Soc., 82, 2077.

Hussain, R.H., 1987, MSc Thesis, University of Bath

Ison, R.R., 1970, PhD Thesis, University of Alberta.

Ison, R.R. and Casy, A.F., 1971, J. Pharm. Pharmacol., 23, 848.

Ison, R.R, Franks, F.M. and Soh, K.S., 1973, J.Pharm. Pharmacol., 25, 887.

James, M.N.G. and Williams, G.J.B., 1974, Can. J. Chem., 52, 1872.

James, M.N.G. and Williams, G.J.B., 1974, ibid, 52, 1880.

Jarrousse, M.J. and Regnier, M.T., 1951, Ann. Pharm. Fran., 9, 321.

Karnes, H.T. and Sarkar, M.A., 1987, Pharm. Res., 4 No 4, 285.

Klemm, L.H. and McCoy, D.R., 1969, J. Heterocyclic Chemistry, 6, 73

Kongo Yakukin K.K., 1976, Chem. Abs., 85, 177255t

Kulrestha, V.K., Gupta, P.P., Turner, P. and Wadsworth, J., 1978, Brit. J. Clin. Pharmacol., 6, 25.

Lehmann, G., 1948, J. Pharmacol. Exp. Ther., 92, 249.

Levander, S, Hagermark, O and Stahle, M., 1985, Eur. J. Clin. Pharmacol, 28, 523

Lim, H.K., Sardessai, M., Hubbard, J.W. and Midha, K.K., 1985, J. Chrom., 328 , 378.

Lochmuller, C.H. and Souter, R.W., 1975, J. Chrom., 113, 283.

Martindale, 1982, The Extra Pharmacopoeia, 28th Edition, The Pharmaceutical Press, London, 1294.

Mercer, A.D., 1988, J. Pharm. Pharmacol., 40(suppl), 126P

Mislow, K. and Raban, M., 1967, Topics in Stereochemistry, Vol 2, 199.

Nakajima, T., Sunagawa, M., Hirashi, T. and Fujioka, K., 1984, Chem. Pharm. Bull., 32 No2, 383.

Nambu, N., Kituchi, K., Kituchi, K., Takahashi, Y., Ueda, H. and Nagai, T., 1978, Chem. Pharm. Bull, 26, 3609.

Nauta, W.Th. and Rekker, R.F., 1978, Handbook of Experimental Pharmacology, Vol XVIII/2, Histamine II and Antihistamines, Silva M.R.e. Ed, Springer Verlag, Berlin, 215.

Nicholson, A.N., 1983, Lancet, July 23, 21.

Nicholson, A.N., 1979, Brit. J. Clin. Pharmacol, 8, 321.

Nicholson, A.N. and Stone, B.M., 1982, Brit. J. Clin. Pharmacol, 13, 289

Niemegeer, C.J.E., Awouters, F., Van Neuten, J.M., De Nollin, S. and Janssen, P.A.J., 1978,Arch. Int. Pharmacodyn., 234, 164.

Novak, L. and Protiva, M., 1959, Coll. Czech. Chem. Comm., 24, 3966.

P'An, S.Y., Gardocki, J.F. and Reilly, J.C., 1954, J. Amer. Pharm. Assoc., 43, 653.

Patent, 1960, B.P. 834,984, Scherico Ltd, Switzerland.

Patent, 1962, B.P. 905,993, M^{c}Neill Labs Ltd, Chem. Abs., 58, 5043g.

Patent, 1978, French Patent 77,15612, Astra Pharmaceuticals, Sweden.

Patent, 1984, U.S.P. 4,539,399, Advanced Separation Technology, U.S.A.

Paton, W.D.M., 1951, Brit. J. Pharmacol., 6, 499.

Peck, A.W., Fowle, A.S. and Bye, C., 1975, Eur. J. Clin. Pharmacol., 8, 455

Pettersson, C. and No, K., 1983, J. Chrom., 282, 671.

Pettersson, C. and Schill, G., 1981, J. Chrom., 204, 179.

Pettersson, C. and Stuurman, H.W., 1984, J. Chrom. Sci., 22, 441.

Pirkle, W.H. and Finn, J.M., 1982, J. Org. Chem., 47, 4037.

Pirkle, W.H., Finn, J.M., Schreiner, J.L. and Hamper, B.C., 1981, J. Amer. Chem. Soc., 103, 3964.

Pirkle, W.H. and House, D.W , 1979, J. Org.Chem., 44 No 12, 1957.

Pirkle, W.H., House, D.W. and Finn, J.M., 1980, J. Chrom., 192, 143.

Rao, V.S.R. and Foster, F., 1963, J. Phys. Chem., 67, 951.

Reinhardt, D. and Borchard, U., 1982, Klin. Wochenshr., 60, 983.

Rekker, R.F., Timmerman, H., Harms, H.F. and Nauta, W.Th., 1971, Arneimittell Forsch, 21, 688.

Rekker, R.F., Timmerman, H., Harms, H.F. and Nauta, W.Th.,1972, Chim. Therap., 7, 279

Roszkowski, A.P. and Govier, W.M., 1959, Pharmacologist, 1, 60.

Roth, F.E., 1961, Chemotherapia, 3, 120.

Roth, F.E. and Govier, W., 1958, J. Pharmacol. Exp. Ther., 124, 347.

Salvadori, P., Rosini, C. and Bertucci, C., 1984, J. Org. Chem., 49, 5050.

Schild, H.O., 1947, Br. J. Pharmacol., 2, 189.

Schild, H.O., 1949, ibid., 4, 277.

Schill, G., Wainer, I.W. and Barkan, S.A., 1986[1], J. Lig. Chrom., 9, 641.

Schill, G., Wainer, I.W. and Barkan, S.A., 1986[2], J. Chrom., 365, 73.

Shafi'ee, A. and Hite, G., 1969, J. Med. Chem., 12, 266.

Silva, M.R.e. and Antonio, , 1978, Handbook of Experimental Pharmacology, Vol XVIII/2, Histamine II and Antihistamines, Silva, M.R.e., Ed, Springer Verlag, 405.

Souter, R.W., 1985, Chromatographic Separations of Stereoisomers, Boca Raton, Florida, CRC Press Inc.

Stelt, C., van der Heus, W.J. and Nauta, W.Th., 1969, Arneimittel Forsch, 19, 2010.

Stewart, K.K. and Doherty, R.F., 1973, Proc. Natl. Acad. Sci. USA., 70, 2850.

Szetjli, J., 1982, Cyclodextrins and their Inclusion Complexes, Budapest, Akademiai Kiado.

Toldy, L., Vargha, L., Toth, I. and Borsky, J., 1959, Acta. Chim. Acad. Sci. Hungary, 19, 273.

Tscherne, R.J. and Capitano, G., 1977, J. Chrom., 136, 337.

Uekama, K., Fujinaga, T., Hirayama, F., Otagiri, M., Kurono, Y. and Ikeda, K., J. Pharm. Pharmacol., 1982, 34, 627.

Uzan, A., Le Fur, G. and Malgouris, C., 1979, J. Pharm. Pharmacol., 31, 701

Velluz L., Legrand, M. and Grosjean, M., 1965, Optical Circular Dichroism, Principles, Measurements and Applications, Verlag Chemie, Gmb H, Weinheim.

Wainer, I.W., Barkan, S.A. and Schill,G., 1986, LC/GC Mag, 5, 422.

Wainer, I.W. and Doyle, T.D., 1984, Liquid Chromatography HPLC Mag, 2 No 2, 88.

Wainer, I.W., Doyle, T.D., Fry, F.S. and Hamidzadeh, Z., 1986, J. Chrom., 355, 149.

Ward, T.J. and Armstrong, D.W., 1986, J. Liq. Chrom., 9 (2+3), 407.

West, G.B., 1958, Int. Arch. Allergy., 13, 336.

Williams K. and Lee, E., 1985, Drugs, 30, 333

Wren, H and Williams, H, 1916, J. Chem. Soc..

Wood, D.J., Hruska, F.E. and Saenger, W., 1977, J. Amer. Chem. Soc., 99, 1735.

Yoshitomi, 1973, Chem. Abs., 79, 42361.

Yoshitomi, 1975, Chem. Abs., 83., 206105.